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*Master thesis*

Effect of changing environmental conditions  
on the nutrient dynamics of wetland soils  
in the Czech military training area Brdy

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*Anotace:*

The aim of this study was to determine the possible impact of eutrophication on a wetland meadow at Dolejší Padrtský pond in the Brdy military area. Changes in soil and plant chemistry on two transects differing in water regimes were monitored after 60 kg/ha/year of NPK fertilizer addition during two years.

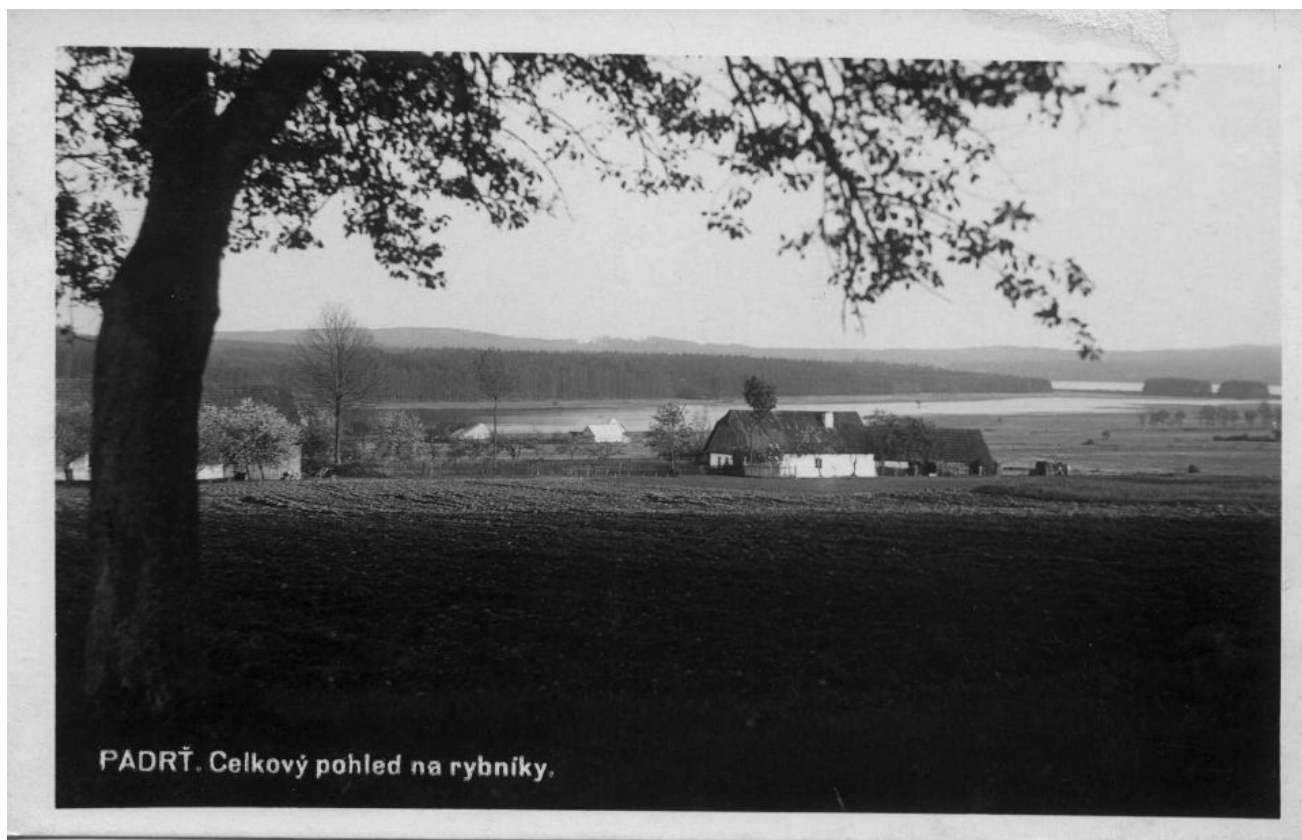
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## Abstract

The aim of this study was to determine the possible impact of eutrophication on a wetland meadow at Dolejší Padrt'ský pond in the Brdy military area.

Changes in soil and plant chemistry on two transects differing in water regimes were monitored after fertilizer addition during two years.

Two factors were considered to impact the nutrient dynamics

- water regime - wet (standing water or water level close to the soil level) and dry (water level under the soil level)

- fertilizer addition of 60 kg/ha/year of NPK fertilizer/year - fertilized and nonfertilized control.

Water regime significantly affected  $\text{NH}_4$ ,  $\text{NO}_3$ , soluble C, total carbon in soil and total nitrogen in plant biomass in the first season. During the second season, the total nitrogen content dynamics in soil was significant. In both seasons, total phosphorus in the plant biomass was significantly affected by the water regime. Organic matter content was higher on the drier transect.

Fertilizer addition significantly impacted  $\text{NO}_3$ , total nitrogen in soil and biomass weight in the first season.

The data showed that there was a difference in the nutrient dynamics caused by fertilizer addition in the first season for  $\text{NO}_3$ , total nitrogen in soil and biomass weight. The influence on other monitored plant and soil properties were insignificant. A correlation between the water levels and the ammonium content was found; the higher was the water level the higher was the ammonium concentration. This was caused by the anaerobic conditions.

Therefore, for the short time of nutrient additions, water regime is considered as an important factor, contrary to the effect of the fertilization doses.

## Introduction

Eutrophication of aquatic ecosystems or ecosystems connected to them can cause an increase in algae and aquatic plant growth, loss of component species and even loss of ecosystem functions due to the nutrient addition (Smith et al. 1999). Eutrophication can modify plants species composition in wetlands of the Northern hemisphere (Morris 1991). It can therefore affect biodiversity (Bollens et al. 2001), usually as the result of differential effects on plant functional groups (Pauli et al. 2002). In studies on the eutrophication of ecosystems, emphasis is placed usually on the relationships between increased nutrient inputs and outputs (Verhoeven 1987). The effect of eutrophication on vegetation has already been studied by many authors (e.g., MacGimmon 1980, Paverly 1982, Hammer and Kadlec 1982). Changes in plant composition are mostly due to changed competitive interactions after nutrient addition (Aerts 1999). Many studies have been done to clarify the mechanisms of nutrient uptake (Pederson 2002). Greater nutrient input levels increase plant tissue nutrient contents. The subsequently decreased C:N ratio in plant tissues results in more rapid decomposition rates of the plant litter and a consequent faster cycling of C and N in the wetland as a whole (Robarts and Waiser 1998). Greater nutrient availability is negatively related to the occurrence of plant mycorrhizae associations (Johnson et al. 2003). Nutrient use efficiencies (NUE) for plants should be greater in nutrient-poor habitats where slow plant growth rates but high nutrient retention rates would be expected (Vitousek 1982).

In most wetland ecosystems, the nutrients taken up by the vegetation originate mainly from the mineralization of nutrients stored in organic matter (Verhoeven 1987). There is a close relationship between biomass production, nutrient demand, and water uptake (Andren et al. 1996). In agricultural soils, mass flow rates of N and K are usually sufficient, but in most natural soils, concentrations of N, P and K are much lower, so that the supply of these nutrients by mass flow alone is insufficient to satisfy the demand. Consequently, diffusion must play an important role in the supply of these nutrients to plants growing on natural soils. This calls for a root system that has the ability to take up nutrients selectively against a concentration gradient (Berendse et al., 1999). The balance between N and P supply, as reflected in N:P ratios of plant biomass, influences the functioning of terrestrial vegetation at all levels; the growth and reproduction of individual plants, plant species interactions, community composition and botanical diversity all depend in a distinct way on the availability of nitrogen and phosphorus to the vegetation. The resulting differences in plant chemistry may determine the activity of herbivores, parasites, pathogens and decomposers (Gusewell 2004).

While the effects of eutrophication on plant communities are relatively predictable, there is greater uncertainty in regards to the responses of soil processes to increased nutrient levels. Organic matter (OM) mineralization rates increase in eutrophic soils, leading to nitrogen and phosphorus release and their enhanced availability for plant growth. Thus, input of eutrophic waters from external sources may increase or accelerate internal eutrophication of an ecosystem (Koerselman et al. 1993). Soil type can affect how soil processes are changed by eutrophication, but there is still great uncertainty in how transformation processes are affected by increased nutrient levels in wetlands with different soil types (de Vries et al.

2003). In general, retention of N, P and K is greater in peat soils (Verhoeven 1986), while mineral soils can sometimes act as sinks for P (Masscheleyn et al. 1992).  $\text{NH}_4^+$  and  $\text{NO}_3^-$  are retained to a much greater extent in peat than mineral soils (Fenn et al. 1998). The amount of nutrients sequestered in soils is affected by OM decomposition rates.(reference)

Agriculture and urban activities are major sources of phosphorus and nitrogen to aquatic ecosystems (Carpenter et al. 1998). Agricultural fertilizers are frequently used in higher doses than what is necessary for plant nutrition. This is because a part of the nutrients are lost from the system, by sorption, leaching, vaporization and immobilization in organic biomass. Long term use of large fertilizer doses has generated negative effects, such as water pollution and health problems due to high nitrogen content in food and drinking water. (Šimek, 2004)

Tourism is another source of pollution which has not been studied properly yet. Millions of people travel to see and experience natural environments each year and the scale of such movements leads, inevitably, to some disturbance or damage to the visited sites. While such damage is attributable directly or indirectly to tourists and their activities, it is often unclear whether their actual behaviour is responsible for the major negative impacts on nature (Deng 2002). Whatever the true situation, it is tourists who are usually identified as causing destruction, particularly in developing countries. Lea (1988) and Olindo (1991) showed how their large volume, demanding access to game, relatively luxurious travel and accommodation facilities, has caused problems such as overcrowding, animal disturbance, vegetation degradation, soil compaction, and waste production (cited in France 1997: 13). Increased tourist activity could lead to increased nutrient additions to the area, resulting in possible future eutrophication (Hadwen 2003).

This study was undertaken to determine the influences of nutrient addition on an unmanaged wet meadow situated in a military area. It is an initial investigation of possible future nutrient additions with a long-term aim of helping to develop a sustainable management plan for this area. The surrounding area of the ponds may be used as a recreation site, which could lead to cottage-building. If this occurs, it will be necessary to solve the waste water treatment.

The study site has not been influenced by eutrophication from agricultural management since the 1940s. This locality is nowadays inaccessible to the public, with the exception that some roads are open for cycling on weekends. In 2005, the Czech Ministry of Defense carried out an analysis concerning the necessity of all Czech military areas to gain base data for this discussion, and to determine if some parts of these areas may be possibly opened to the public. Results showed that some parts of the Brdy Military area might be opened to the public (Anonymous, 2005). The particular meadow chosen for this study was a frequently visited place in the past, mainly for recreation and as a swimming site, until 1945 (Čáka 2003).

For this study, the following aims and hypothesis have been set:

### **Aims of the study:**

To describe the present nutrient levels in a low-disturbance (unaffected) wetland in a military area.

To determine possible impacts of increased disturbance, in the form of eutrophication due to fertilization, on wetland soil and plant nutrient dynamics, if the military area is opened to private

development. This study should serve as an information base for management proposals, pro-active management propagation, or more detailed, specialized research projects.

### **Hypothesis:**

- Short-term nutrient addition will lead to increased plant nutrient uptake and decreased nutrient use efficiency
- Change in nutrient dynamics in the soil will be lower in the wetter areas of the study site; this should be caused by greater water level fluctuations.

### **Site description:**

The Military area Brdy is situated among the cities Příbram, Rokycany and Hořovice in western Bohemia, with a total area of 260,34 km<sup>2</sup>. Habitat types in the Brdy area include forests, heathlands, ponds and wetlands. Brdy Military area is an important water source for western and central Bohemia and was declared a Protected area of natural water accumulation (CHOPAV; Hošnová 1995).

The military area has been unaffected by usual human activities, such as agriculture management, industry or tourism, since 1945. This has led to the emergence of new refuges for many threatened species of plants and animals.

The area was proposed as a National park in 1920. In 1924 this proposal fell through when the military area was opened, Many famous personalities, such as the botanist Karel Domin and the poet Viktor Dyk, officially protested against it (Čáka 2003). A new wave of calls for proclaiming this region as a protected area started again in 1991 (Anonymous).

A study called Projekt Brdy was published in 2005 (Čámský 2005), showing the requirements and wishes of the local people to open the area. Three scenarios were presented concerning possible economic activities in the opened area, including different types of tourism.

The United States of America showed interest in building a radar base on a neighbouring hill. The final resolution was to change the site and build the base in Poland.

The study was conducted on a meadow on the western shore of Dolejší Padrt'ský pond. The submountainous Padrt'ské ponds are situated in the south-western part of the military area Brdy. They form a two pond system; Hořejší pond is connected by a brook to Dolejší Padrt'ský pond, which serves as an important source to the Padrt'ský brook, which is one of the tributaries of the Berounka river.

The altitude of Dolejší Padrt'ský pond is 635 m above sea level; it is the fifth highest pond in altitude in the Czech Republic. The pond is surrounded by coniferous forests on the east and wet meadows on the west. The free water area of the Dolejší pond is 39,025 ha.(Hošnová 1995)

There is a continuous change from the pond littoral to the wet and then drier meadows. On the southern part of the pond, there is a small island which is important for waterfowl. There are springs on the pond bottom. The watershed is 4,879 km<sup>2</sup> according to the State hydrological institute T.G.M. in Prague.

This area is classified as a CH7 climate region, with a very short slightly warm summer and a relatively long winter (Quitt 1971). The average annual temperature is 5-7°C.



Soil type of the studied site is gley soils (Chlupáč et al. 1994?). Černý et al. (1991) found that the mountains in the surrounding forests are influenced by acid rain, with average pH (KCl) of 3,16 in the humus layer and 3,25 in the mineral layer. This was confirmed by Stuchlík et al.(2000).

### **Geologic substrate**

A border of algonic and cambrian substrates goes through the surroundings of the ponds. The origin of the pond is unclear; there was probably an old sea basin, which was sometimes flooded to a much higher level (Jahn, 1925). The base of the pond is composed of azoic slate, which agglomerates above it partly with a sandy alluvium, although with peat in some places (Domin, 1903). Klečka (1926) and Firbas (1927) claim that probably only Hořejší pond is of a lake origin; according to them it is an old peat bog. Cílek 1993 published more data about the site geomorphology.

### **Management**

Fish are kept in the pond and are harvested every three years; the management of the pond is extensive. The pond is regarded as being slightly eutrophic, IV. -V. class, which means weak to very weak growth figure (0-50 kg) (Sládeček 1949). More current data are not available. Earlier, Kafka (1892) criticized the use of carp in this pond and stated that trout should be kept instead. The main use of the ponds is for water retention.

### **Vegetation**

The following species were found on the studied sites: Drier transect: *Carex brizoides* L. - dominant species, very abundant, building a thick layer of old biomass, *Alopecurus* sp., *Anemone nemorosa* L., *Trollius altissimus* L., *Filipendula ulmaria* L., and *Iris sibirica* L. close to the transect - 20 cm.

Wetter transect: *Carex nigra* (L.) Reichard - the dominant species, *Carex vesicaria* L. , *Carex pseudocyperus* L., *Galium palustre* L., *Comarum palustre* L., *Salix caprea* L..

### **History**

The origin of the ponds is unclear. At least one of the ponds had already been created by 1640 (Kudrnovská 1940). The ponds were used in the 16th century by the Griespeks from Mirošov for ferrous metallurgy. Until 1945, the owner of these ponds was Count Colloredo- Mansfeld; since then the manager has been Vojenské lesy a statky s.p. (Military forests and goods, a state company).

There was a village called Padrt' on the northern shore of Dolejší Padrt'ský pond until 1945, but it was destroyed at the beginning of the communist period.

## **Data review**

### **Data concerning the study site**

Many data were collected before or shortly after the conversion of the area to military purposes. Josef Kafka investigated plankton in 1892, Julius Komárek studied invertebrates in 1925, and Karel Rosa algae in 1939, with special emphasis on *Desmidiaceae*. Vegetation surveys were conducted by Karel Domin (1903 -

1926) and Karel Cejp (1924, 1925). Water fungi and *Sphagnum* mosses were studied by Kleka (1926) and Firbas (1927).

In 1949 Sládeček described the chemical properties of the ponds:

	Water transparency	Phosphates	Conductivity
Hořejší pond	205 - 60 cm	0,14 mg/l	58,1 * 106 1/Ω
Dolejší pond	140- 60 cm	0,23 mg/l	54 * 106 1/Ω

Nowadays, after the change in 1989, studies in this area are possible again. These include vegetation studies (Sofron 1998), and a common overview (Němec 1998). The publication Květena Brd (Flora of Brdy mountains), which should summarize the vegetation in the Brdy area, should be published in the next few years. (Hlaváček et al., prepared)

### **Carex brizoides**

*Carex brizoides* grows in southern and central perimontaneous areas of central European (it is completely missing in the eastern part of the Sarmatian province and is rarely present in the Atlantic province) (Meusel & Buhl 1968). It prefers altitudes between 700 and 900 above sea level.

*Carex brizoides* grows on heavier, compacted, mostly gleyed soils, which are wet or at least water logged, but do not dry out. It often creates large growths on soil with a high water level. It is often found on wet locations near forest brooks, and in wet forests, but also on meadows from lowlands to mountains (from oak woods to beech-spruce forests). It can overgrow unmanaged alluvial meadows and wetter slopes, mostly on higher altitudes and acid soils. Vegetation with *Carex brizoides* is species poor and may persist for tens of years. It is a clonal, vegetatively expanding species (Vacková 1997). It is frequently observed that the number of such species increases in meadows where mowing has been stopped (Falinska 1991). Cattle do not graze on it. Except for sheep, it can not be used as a fodder crop. The only advantage of this species is that its presence probably maintains forest free areas. The most frequent species growing together with *Carex brizoides* are *Filipendula ulmaria* L., *Caltha palustris* L., and *Carex rostrata* Stokes (Husáková not published). A stochastic - deterministic model of the growth of this plant showed a growth after 12-14 years of the cultivation. (Tumidajowicz 2006)

## **Materials and methods**

### **Research conditions**

#### ***Experiment details***

This study compares soil and plant chemistry on two sites in the military area Brdy. Studied sites differ in water regimes and were treated with nutrient addition of 60 kg/ha/year of NPK fertilizer. Therefore, the effect of eutrophication was monitored and compared between two transects over two growing seasons.

The wetter transect, with higher groundwater level, was established at a distance of 5 meters from the free water area of the pond. The drier transect, with lower groundwater level, was located 80 meters from the free water area of the pond. Each transect ran parallel to the pond edge and consisted of eight 1x1m plots with 2 m buffer zones between plots to prevent fertilizer contamination on control plots (see below).

In each transect, four plots were treated with fertilizer addition and four plots acted as controls (without added fertilizer). The establishment of a particular treatment (fertilized or control) was done randomly for each transect.

The experimental design is shown in Figure 21. in the appendix.

#### ***Fertilizer***

A 11-7-7, universal NPK fertilizer (type number 5.1, registration by ÚKSÚP: 207/2002. Producer: Lovochemie a.s. Městec Králové, CZ) was added in the total amount of 60 kg/ha/year. Particular portions were added through the growing season every time after sampling.

#### ***Groundwater level***

Ground water level was measured on every sampling date. Ground water level was measured with a ruler placed into a small-diameter hole, taking into account the distance from the soil surface to the water surface.

#### ***Soil properties***

The following soil properties were measured in each plot (three samples were taken in each plot from different randomly chosen 10x10 cm squares):

soil organic matter content - once at the begin of the study,

NH<sub>4</sub>, NO<sub>3</sub>, total nitrogen, carbon and phosphorus (TN, TC, TP) and soluble carbon - three times per season.

Soil samples were stored for a week in a cold dry place. After this time, the samples were analyzed as described below. Water samples were analyzed as soon as possible after collecting the samples.

### **Plant properties**

In each plot, dry weight of each plant species, as well as TP in biomass, were measured in September 2005 and 2006. Three samples were taken from different randomly chosen 10x10 cm squares in each plot at each sampling date.

Net aboveground production was estimated using a production to biomass ratio for *Carex brizoides* and also *Carex nigra* (the two dominant species in the dry and wet plots respectively). Ratios of annual net production to maximum biomass - P/B ratios were used 1.15 for *Carex brizoides* and 1.3 for *Carex nigra* was used - 1.5. These ratios were estimated according to other literature (Rychnovská 1985) and to the fact that both dominant species have a lot of standing stock and their decomposition is very slow. The maximum biomass has been extrapolated to 1m<sup>2</sup> and multiplied by the P/B ratio.

Nitrogen use efficiencies (NUE) were calculated for the different treatments based on the method of Lopez-Bellido and Lopez-Bellido (2001). Briefly, bulk density was multiplied by the volume of the soil, where the samples had been collected (10cm \*100cm \*100cm). To get the nitrogen supply, NO<sub>3</sub> concentration from the first sampling in the monitored year was transformed to mg/g and the amount of the added fertilizer was added. Nitrogen use efficiency in the monitored year was calculated as the plant biomass divided by the N supply for that date.

## **Chemical analysis**

### **Organic matter content**

Five samples were taken from each plot using a corer (20 cm length and 5 cm diameter). All samples were mixed together. From this, a 10 g sample was exactly weighed and dried at 50° C for 12 hours, burnt in a muffle oven at 450° C for 2 hours and weighed again. Organic matter content is the difference in the weight before and after burning. The results are shown in percentage of organic matter in the sample.

### **Soil water nutrients**

NH<sub>4</sub>, NO<sub>3</sub>, soluble C, TP

On the wetter transect, 1m deep tubes were put into each plot. Ground water remaining in the tubes was collected and filtered through a paper filter. A soil water solution was made from the soil taken from the drier site: 10 g of sample was weighed into an NTS bottle and 40 ml of distilled water was added. The suspension was shaken for 45 minutes and filtered through a paper filter, which had been rinsed out with hot water before to remove any excess ammonia. N - NO<sub>3</sub> and NH<sub>4</sub> of the filtrates were measured on a FIAstar 5012, Detector 5042 (Foss, Sweden).

Principle of FIA:

Mineralized forms of nitrogen, phosphorus and other ions and compounds in water, soil extracts and other liquid samples can be analyzed using a FIA. The sample is mixed with a reaction chemical, causing a tinge, whose intensity is measured by a spectrophotometer.

The sample is sucked from the autosampler into a dosing valve. A peristaltic pump pumps an exact volume into the chemifold through hoses. In the chemifold, the sample is mixed with chemicals in the same ratio. The mixture flows through a cuvette, where the absorbency is measured. From a calibration curve, the concentration of the concrete ion in the sample can be calculated.

The concentrations of  $\text{NH}_4\text{-N}$ ,  $\text{NO}_3\text{-N}$ , dissolved inorganic nitrogen (DIN), and dissolved reactive phosphorus (DRP), were determined in water filtered through Whatman GF/C filters using a Tecator flow injection analyzer. The gas diffusion method was used for the estimation of  $\text{NH}_4\text{-N}$  (Karlberg and Twengström, 1983).  $\text{NO}_3\text{-N}$  was determined as nitrate after reduction of the sample in a Cd-Cu column. The standard phosphomolybdenum complex method was used for the DRP estimation (Parsons et al., 1984). The total nitrogen (TN) and total phosphorus (TP) values were determined as  $\text{NH}_4\text{-N}$  and DRP respectively, after mineralization of samples by persulfate.

A KCl soil solution was prepared for the soluble carbon analyses: 10 g of sample was weighed into an NTS bottle and 40 ml of 2M KCl were added. The suspension was then shaken for 45 minutes and filtered through a normal paper filter. All prepared samples were then analyzed on the FIAstar 5012.

### **Total N and C**

Samples were dried for 8 hours at 60°C and ground at 30 revolutions per second for 2 minutes on a mill (Mixer Mill MM 200, Retsch, Germany). A sample of about 3 µg was measured and then analyzed on an NC 2100 Soil Analyzer (ThermoQuest Italia S.p.A., Italy).

Principle: completely oxidized by combustion, organic nitrogen is converted into elemental nitrogen and carbon into carbon dioxide. The gas mixture is then separated on a gas chromatographic column and measured using a thermoconductivity detector.

### **Statistics**

Data describing the soil properties were analyzed by nested ANOVA. The factor Fertilizer was nested in the moisture factor (wet/dry). Repeated measures had to be added in the syntax window, depending on the frequency of sampling (2 - 6 times). A single test was made for each season. Data from the May 2005 sampling date were used as covariates to enable the comparison of the dry and wet sampling sites. A multivariate test of significance was performed on plant dry weight, while a univariate significance test was used for organic matter content. Multiple regression was used to analyze the relationship between water level and  $\text{NH}_4$  concentration. Statistica 6 (Moore 2007) was used for all analyses.

## Results

### Soil properties

#### *Water levels*

Water levels in the wet plots ranged between -5 to + 15 cm above the soil level, while in the drier part the water level was about -10 to - 30 cm below soil level.

#### *Nitrogen*

At the beginning of the study in May 2005, the concentrations of N-NH<sub>4</sub> varied from 0,43 to 0,68 mg/l on the wet transect with an average of 0,54 mg/l and varied from 0,62 to 0,8 mg/l on the drier transect with an average of 0,71 mg/l. Statistical tests showed a significant difference between these two transects at this time as well as in the other sampling times, with the exception of the last one in July 2006. This could have been caused by the weather; the wetter transect was standing out of the water at the time of the sampling.

The greatest difference was in September 2005, with average N-NH<sub>4</sub> concentration of 0,4 mg/l on the wet transect and 0,86 on the drier transect. At this time, the wetter transect was completely waterlogged. See the appendix for the NH<sub>4</sub> dynamic graph (figure 2).

Ammonium concentrations were higher on the fertilized plots but these were not significant (figure 1). In 2006, the differences were much lower. There was no difference in NH<sub>4</sub> concentrations between fertilized and unfertilized plots at the beginning of the study in May 2005.

Figure 3 shows that there was a trend of higher mean ammonium content in higher water levels on the wetter sites. These differences were significant ( $p < 0,029$ ,  $R^2 = 0,714$ ).

Concentrations of NO<sub>3</sub> ranged from 0,07 to 0,39 mg/l. In 2005, nitrate concentrations were statistically different for both factors: fertilizer addition and wetness of the plot. There were no significant changes over time for all of the factors. The highest average concentration was in 2005 on the dry plots (Figure 6). In 2005, nitrate concentrations increased from May to July in the fertilized plots and then decreased by September (figure 5). On nonfertilized plots, there was no decrease of NO<sub>3</sub> from July to September 2005. Fertilization ( $P < 0,638$ ) and wetness ( $p < 0,097$ ) of the plots did not significantly impact NO<sub>3</sub> concentrations in 2006. In both years the concentrations of NO<sub>3</sub> increased from May to July sampling on all plots.

TN concentrations ranged from 0,32 to 1,6 %. Concentrations of TN on plots with fertilizer addition were significantly higher in the first season ( $p < 0,012$ : figure 7). This difference was much lower in the second year, and not statistically significant. In the second year (2006), TN was significantly higher on the dry plots ( $p < 0,011$ , figure 8). The dynamics of TN were significantly different on the wet and dry plots in the second season ( $p < 0,002$ ). No other time intercepts were significant during both seasons.

### **Carbon**

Concentration of soluble carbon ranged from 2,6-18,7 mg/l and was significantly different in the wet and dry plots during the first year being higher in the wet plots (figure 9). There was a weakly insignificant fertilizer effect in the first year, the concentrations were similar in May and diverse in 2006. The concentrations show a general tendency in their dynamics during the season, being highest in May and then decreasing from July to September (figure 10). The data values increased again at the beginning of the new season.

Total carbon ranged from 3.4 to 28.1 %. There was a significant difference between the wet and dry areas in total carbon concentrations In 2005, being higher on fertilized dry plots (figure 11). The effect of fertilizer was insignificant in both seasons. The highest concentrations were generally in September 2005 and July 2006 with the exception of the wet plots, where the values were highest in July of the first year (figure 12). TC changed significantly over the growing season; in the first year there was a significant difference in the dynamics between the wet and dry plots, whereas this was not significant in the second season.

### **Organic matter content**

Organic matter content was significantly higher on the wetter plots (figure 24). Mean was 29 % for the wet plots and 48 % for the dry plots. There was no difference between the fertilized and non-fertilized plots (figure 23).

## **Plant measures**

### ***Total phosphorus in plant biomass***

The concentration of total phosphorus in plant biomass was significantly different in wet and dry plots in both seasons (figure 14) with concentrations being lower in the drier site (figure 13). There were no significant differences between fertilized and control treatments. The data ranged from 1.02 to 1.72 mg/g and were higher in 2005 than 2006.

### ***Total nitrogen (TN) in plant biomass***

The concentration of total nitrogen in plant biomass ranged from 1,02 to 1,72 mg/g and was significantly different in wet and dry plots in the first year with concentrations being lower on the drier site (figure 16). Plant TN did not differ between the treatments in either season, although that, the TN content was higher in the nonfertilized plots in the second year (figure 15).

### ***Dry biomass weight***

Dry biomass ranged from 114 to 279 g/m<sup>2</sup>. Dry weight was significantly higher in the fertilized plots in both years - 2005 and 2006. Biomass in the fertilized plots was lower in 2005 than in 2006 (figure 21). This was also the case for the wet plots (figure 22), however, this difference was not significant -  $p < 0,069$ .

### ***Net aboveground production***

In 2005, net aboveground production ranged from 150 to 540 g/m<sup>2</sup>, in 2006 the data ranged from 130 to 403 g/m<sup>2</sup>. The effect of the fertilizer addition was insignificant for both seasons, although production was higher on the fertilized plots in both years (figure 17). This difference was larger in the first year.

The net aboveground production differed significantly in both seasons depending on the wetness of the plots ( $p < 0,012$ ) (figure 18). In 2005 the NAP was on average 256 g/m<sup>2</sup> on wet and 220 g/m<sup>2</sup> on dry plots, but, the values were opposite in 2006- 200 g/m<sup>2</sup> on wet and 279 g/m<sup>2</sup> on dry plots.

### ***Nitrogen use efficiencies***

Nitrogen use efficiencies ranged from 8,3 % to 14 % (mean 10,71 %) in 2005 and from 6,91 % to 36,6 % (mean 17,8 %) in 2006. In both years, the differences for both fertilizer addition and wetness of the plot were insignificant. In 2005, the average nitrogen use efficiencies were 10,5 % on the fertilized plots and 10,9 % on the nonfertilized plots (figure 19), and 11,08 % on wet and 10,3 % on dry plots. In 2006 the mean was 19,9 % on the fertilized and 16,4 % on the nonfertilized plots and 16,8 % on wet and 18,8 % on dry plots (figure 20).



## Discussion

This study compares soil and plant chemistry on two sites in the military area Brdy. The study sites differ in water regimes and were treated with nutrient additions of 60 kg/ha/year of NPK fertilizer. Therefore, the effect of eutrophication was monitored and compared between two transects over two growing seasons.

### *Effect of fertilizer addition*

A difference in nutrient dynamics after fertilizer addition was found for NO<sub>3</sub>, total nitrogen in soil and plant biomass in the first season. The influence of the fertilizer addition on other monitored plant and soil properties was insignificant. The TN content was higher in the nonfertilized plots in the second year, but the difference was statistically insignificant.

### *Differences between wet and dry plots*

The collected data show that the most important factor that influenced the nutrient dynamics was the wetness of the plot. This factor was significant in the first season for NH<sub>4</sub>, NO<sub>3</sub>, both soluble and total carbon in soil and total nitrogen in plant biomass. Differences for total nitrogen in soil between the wet and dry plots were slightly non-significant ( $p < 0,065$ ). In the second season, total nitrogen in soil was significantly higher on dry plots. The nutrient dynamics of total phosphorus in the plant biomass were significantly affected by wetness in both seasons. Wetness also played an important role in organic matter content, which was higher on the drier site.

### *Ammonium content*

Water levels and ammonium content were correlated: the higher was the water level the higher was the ammonium concentration. This was likely caused by the presence of anaerobic conditions. Decomposition is faster under continuous aerobic conditions, although inorganic nitrogen is usually released earlier to the soil solution under anaerobic conditions. Under certain conditions, organic nitrogen is converted to ammonium. This process occurs in both aerobic and anaerobic soils; under aerobic conditions the ammonium is oxidized to nitrate while under anaerobic conditions the nitrate is denitrified (Reddy, 1975). Sahrawat (1980), in experiments with alternate flooding and drying, showed that flooding and drying treatments retarded nitrification of soil N but conserved fertilizer NH<sub>4</sub><sup>+</sup> applied after these treatments. A similar relation has been described by other authors e.g. Monaghan (1994) or Vymazal (2003).

The difference in the measured data was partly caused by the dissimilar climate conditions in the monitored years. Data obtained from the Czech Hydrometeorological Institute (CHMI) show that there was more precipitation, with less sunshine, and cooler conditions on the study site in 2005 than 2006 ([www.chmi.cz](http://www.chmi.cz)).

It is possible that the effect of fertilization would arise after some years of continual nutrient addition, because the nutrient input was not very high and the time was relatively short. The effects of long-term nutrient additions were studied by many authors (CITATIONS) and they basically show a delay in the effect

of phosphorus and nitrogen addition on biomass and species composition by the amount of added fertilizer used in this study. For example, van der Hoek, et al (2004) studied nutrient limitation and nutrient-driven shifts in plant species composition in a species-rich fen meadow. They observed fast immobilization and subsequent slow release of fertilizer P in the soil. Recurrence of P pulses is therefore generally expected to cause permanent changes in species composition.

Gusewell et al. (2003) studied the reactions of 16 wetland plant species to nitrogen and phosphorus additions and different water regimes. They combined three nutrient levels (low N and P, high N, high P) with three water regimes (constantly wet, periodically aerated, periodically flooded). Total biomass produced during both monitored years was smaller at high N than high P supply. Periodic flooding reduced biomass production and nutrient recovery, but hardly influenced the effects of nutrient supply on plant growth (Gusewell et al. 2003). These facts come out also in this study: flooding had a negative effect on plant growth in the second year. These results show that not only N and P enrichment may have quite different effects on wetland vegetation (Gusewell et al. 2003), but also different water regimes are important also due to the changing climate. These results suggest the necessity of using different water regimes in eutrophication simulations.

In 2007 and 2008, Edwards et al. (unpublished data) carried out fertilization experiments on two wetland sites in the Třeboňsko Basin Biosphere Reserve (TBBR). The first, Zábłatské Louky (ZL), is on peat soils while the second is on a silt-sand alluvial substrate and is located near the village of Hamr (H). The altitude is 426 m above sea level for ZL and 415 m above sea level for the H site. Carbon accumulation is typical of the poorly flushed marginal littoral zones of shallow reservoirs such as fishponds in the TBBR. The Zábłatské Louky site is a marginal wetland (Prach 2002), located in the inundation area of a large fishpond. As the water level in the fishpond is kept within a narrow range throughout the year, also the water level is usually fairly stable in the adjacent Zábłatské Louky wetland (except for long periods of extreme summer drought and several weeks long periods of fishpond drawdown in autumn). The Hamr site is located in the floodplain of a small river (Nežárka) and the water level is the same as in local drainage ditches connected with the river. Hence, the water level is more variable here than in Zábłatské Louky. However, the average water level is lower in the floodplain site (H) than in the marginal fishpond littoral (ZL).

The ZL site is a sedge meadow dominated by *Carex vesicaria* and *C. acuta*, while *Glyceria maxima* and *C. acuta* are dominant in the H site. Both soils in the two study sites are classified as silty-clays; the other physical and chemical parameters are shown in Table 1.

Nitrogen and also carbon content show very similar trends on Zablatske Louky, Hamr and the wet meadow by the Padrtský pond - see appendix - figures 25-28. The lower fertilizer addition, which was the same amount as used in this study, did not have a significant effect on the soil chemistry. Higher doses used in ZL and H had a much bigger effect. Plant dry weight in the Padrtský pond meadow is similar to the Hamr site, being higher in the second year.

Site	H	ZL
bulk density [g.cm <sup>-3</sup> ]	0.52 ± 0.04	0.21 ± 0.02
total C [%]	9.63 ± 1.65	22.33 ± 2.25
total N [%]	0.64 ± 0.1	1.18 ± 0.09
total P [%]	0.18 ± 0.02	0.19 ± 0.01
C to N ratio	15.0	18.9
pH <sub>H2O</sub>	4.9	5.1

Table 1. Physical and chemical parameters of soils at Hamr (H) and Zábřatské Louky (ZL) study sites (from Pícek et al. 2008).

### Nitrogen use efficiency

Because the studied *Carex* species are the dominant species, comprising a large proportion of the total plant biomass, as a result, they determine to a large extent the nitrogen use efficiency at the community level. Nitrogen use efficiency (NUE) is usually measured as productivity per unit nitrogen uptake or loss (Vitousek 1982). Aerts et al. (1994) determined that nitrogen use efficiency increases with increasing maximum growth rate. This increase is due to an interspecific increase of the mean residence time of nitrogen with increasing maximum growth rate and is not caused by an interspecific increase of N productivity.

One of the hypotheses of this study was that, after nutrient addition, nutrient uptake efficiency would be lower. In this study, we did not measure the plant properties at the beginning of the season. Therefore, it is not possible to tell if the nutrient use efficiency was higher or lower. However, knowing soil nitrate concentrations from the beginning of the study (uninfluenced by the treatments), it was possible to create a general scheme of nitrogen turnover cycle as it is shown in the following scheme (Diagram 1):

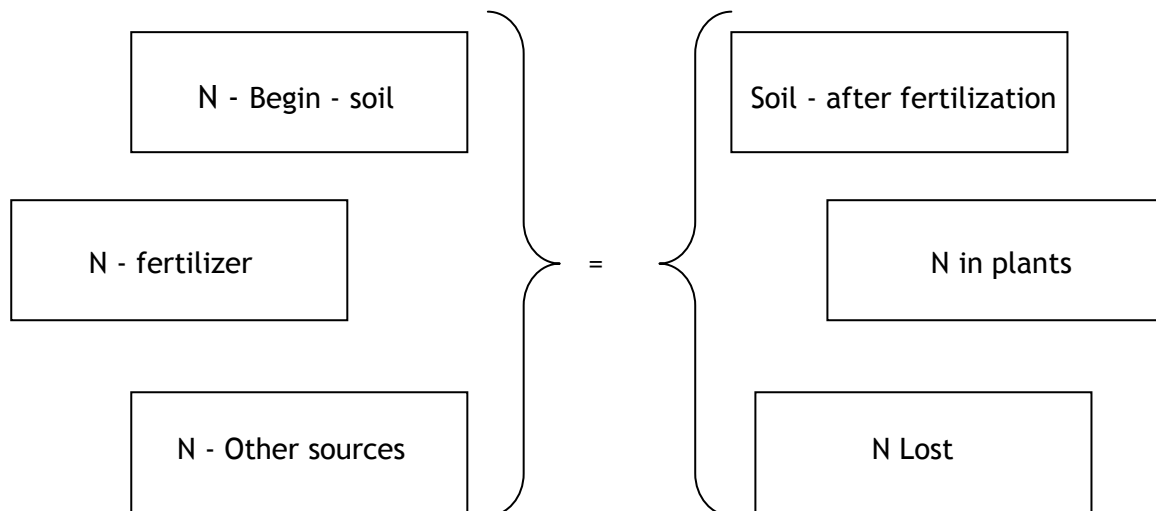


Diagram 1: Nitrogen balance

Nitrogen concentration in the soil at the beginning of the study (nonfertilized) + nitrogen gained from the fertilizer + nitrogen from other sources (atmosphere etc.) should be similar to total nitrogen in the soil after fertilizing + total nitrogen in the plant biomass + nitrogen lost (leaching, volatilization..). This scheme can be used for estimating possible eutrophication processes, by estimating the rates and pathways.

The nitrogen-use efficiency of species at constant levels of nutrient supply tends to increase with increasing nutrient availability in their preferred habitat, according to the Clansman nutrient index (Clausman 1987), up to a certain nutrient level and then decreases. The results support the contention that species from nutrient-poor sites are not necessarily adapted to living in these less-than-optimal conditions due to a high nitrogen-use efficiency, but by low nutrient loss rates (high mean residence time of N in the plant; Vazquez de Aldana and Berendse 1997).

In this study, after nutrient addition, nitrogen efficiency slightly decreased in the first year and increased in the second year. Both of the TBBR study sites (Edwards et al., unpublished data) show a general trend to fall during the growing season with increasing amount of added fertilizer. This indicates the necessity to calculate NUE more times per season. More frequent sampling of plant biomass may be a useful component of future research studies at the studied site.

#### *Net aboveground biomass*

Net aboveground biomass of *Carex brizoides* was also measured by Procházka (2004). This study was done on small stream catchments in the Bohemian Forest. Reported net aboveground production in 2000 was 460 g/m<sup>2</sup>. This is more than the production measured in the Brdy Mountains (average for both years 240 g/m<sup>2</sup>). This can be caused either by a more nutrient rich substrate in the Bohemian Forest or different climatic conditions in 2000, which had more sunny days ([www.chmi.cz](http://www.chmi.cz)).

#### *Study hypotheses*

The following hypotheses were to be tested in this study:

- Short-term nutrient addition will lead to increased plant nutrient uptake and decreased nutrient use efficiency
- Change in nutrient dynamics in the soil will be lower in the wetter areas of the study site; this should be caused by greater water level fluctuations.

Short-term nutrient addition did not lead to increased plant nutrient uptake nor to decreased nutrient use efficiency. The fertilizer dose was probably too low for just two years application. Nutrient use efficiency is changing rapidly during the season, therefore more frequent sampling is necessary to find out the dynamics of these characteristics. Therefore, this hypothesis can not be validated.

However, the second hypothesis can be validated. Change in the nutrient dynamics was lower in the wetter areas.

### **Management recommendation:**

It is necessary that further studies be conducted to determine in greater detail and with a greater level of accuracy the nutrient dynamics of the study site. These will then help in determining which activities or how eutrophication could threaten this ecosystem. Climate change (above all precipitation), resulting in changes to system hydrology, should be monitored and considered by the fishpond management.

*Carex brizoides* is the dominant species on the studied site. This species may suppress other species, thereby threatening endangered species such as *Trollius atlissimus* or *Iris sibirica*.

Blažková (1995) recommended periodical grass mowing to increase the number of plant species. However, mowing often lowers biomass amount, but does not improve biodiversity. Sheep grazing has also been recommended as a tool for reducing the dominance of *Carex brizoides* (Husáková 1995).

Hakrová (2004) found that four years of mowing did not lead to any changes in the dominance of *C. brizoides*. This author came to the conclusion that it is not possible to completely eradicate this species by the above mentioned methods; however, it is possible that some other species may occur on the sites after this management.

Further monitoring of the expansion of this species is recommended; the best method how to eradicate this species is still unknown.

To set out more management recommendations it is important to consider a range of possible scenarios describing how the area may change in the future. The following scenarios are very general; detailed descriptions should be a task of future research.

## Scenarios

The following scenarios give a general overview of what may happen in the area, in terms of the possible impacts on the environment, if it was open to the public:

### 1. No change

This is a baseline scenario where the present conditions continue into the near future (Alcamo 2001). It is not necessary that this site must be rapidly changed anyhow. This would mean that there is only a light form of tourism and the public does not use the area for recreation. It may be that this area is simply too far away for other uses than as an interesting target for bikers. This situation since 2007 is that a bicycle path was opened on the road by the ponds. Nowadays, it is not allowed to leave the marked path.

### 2. Tourism

#### a) only passing by

This scenario represents a very light form of tourism. Tourists are in the area just during the day. It is prohibited to camp on the site or to build any buildings except information boards. Pollution levels are not very high, although there should be litter bins and possibly removable toilets on the most frequent sites. A few public buildings might be built to increase the desirability of the area. If tourists would have the possibility to build any houses in the surroundings of the pond, they might use them in the following ways: 1) As a natural swimming pool; 2) for fishing from the shores; 3) in the winter for skating; or 4) just as a nice place for walking. In addition, some benches or shady constructions, could be constructed.

#### b) Cottages

Many cottages are built in the area. Pollution levels are dependent on the season with peaks occurring on the weekends. It is necessary to build a wastewater treatment facility (very convenient would be a root zone wastewater treatment wetland). Waste has to be removed also with respect to the season. There is a danger of invasive plants spreading from gardens; special containers for garden waste are requested, with education being important.

There will also be pollution originating from transportation, among others by how snow and ice are dealt with during winter. Roads should not be salted, with gravel used instead. The meadow will probably be mown and used for recreation. Special attention should be paid to guarding protected species.

In any case, because of the history of the area, a pyrotechnical remediation must be done on the site.

### 3. A normal residential area

This scenario is not very probable in the near future. The area is not connected to public transportation services and there might be a problem with civil engineering. However, if there was a cottage area, some residential buildings may eventually be built. A canalization net would have to be planned and constructed to prevent pollution from households.

### 4. Agricultural use

It is also possible that the meadow and its surroundings will be used for agriculture. Any agricultural use should be directed towards landscape management, because the *Carex* vegetation is not very appropriate for cattle grazing except of sheep (Vacková 1997). Elimination of *Carex brizoides* would be a good measure for aiding endangered species.

For all scenarios:

Education and cooperation with the local people and tourists are very important and necessary to prevent environmental damage. The education of guests with respect to biodiversity is a key element of environmentally friendly tourism (Waller 2001).

## Summary

This study compared soil and plant chemistry on two sites in the military area Brdy. The studied sites differed in water regimes and were treated with nutrient addition of 60 kg/ha/year of NPK fertilizer. Therefore, the effect of eutrophication was monitored and compared between two transects over two growing seasons.

Fertilizer addition affected the nutrient dynamics of  $\text{NO}_3$ , total nitrogen in soil and plant biomass in the first season. The influence of fertilizer addition on other monitored plant and soil properties was insignificant. TN content was higher in the nonfertilized plots in the second year; however, the differences in this year were insignificant.

The collected data show that wetness of the plot was the most important factor that influenced nutrient dynamics. This factor was significant in the first season for  $\text{NH}_4$ ,  $\text{NO}_3$ , both soluble and total carbon in soil and total nitrogen in plant biomass. The difference between the wet and dry plot was almost significant for total nitrogen in soil ( $p < 0,065$ ). In the second season, total nitrogen in soil was significantly higher on dry plots. The effect of site wetness on the nutrient dynamics of total phosphorus in plant biomass was significant in both seasons.

Water regime also played an important role in organic matter content, which was higher on the drier site. Water levels and ammonium content were positively correlated. This was supposedly caused by the occurrence of anaerobic conditions.

Generally, short-term nutrient addition did not lead to increased plant nutrient uptake nor to decreased nutrient use efficiency. The fertilizer dose was probably too low for just two years application. Nutrient use efficiency changed rapidly during the season, therefore more frequent sampling is necessary to determine the dynamics of this characteristic. The first hypothesis could not be validated.

The second hypothesis was validated; change in the nutrient dynamics was lower in the wetter areas. Management recommendations and future development scenarios were promulgated.

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# Appendix

## N - NH<sub>4</sub>

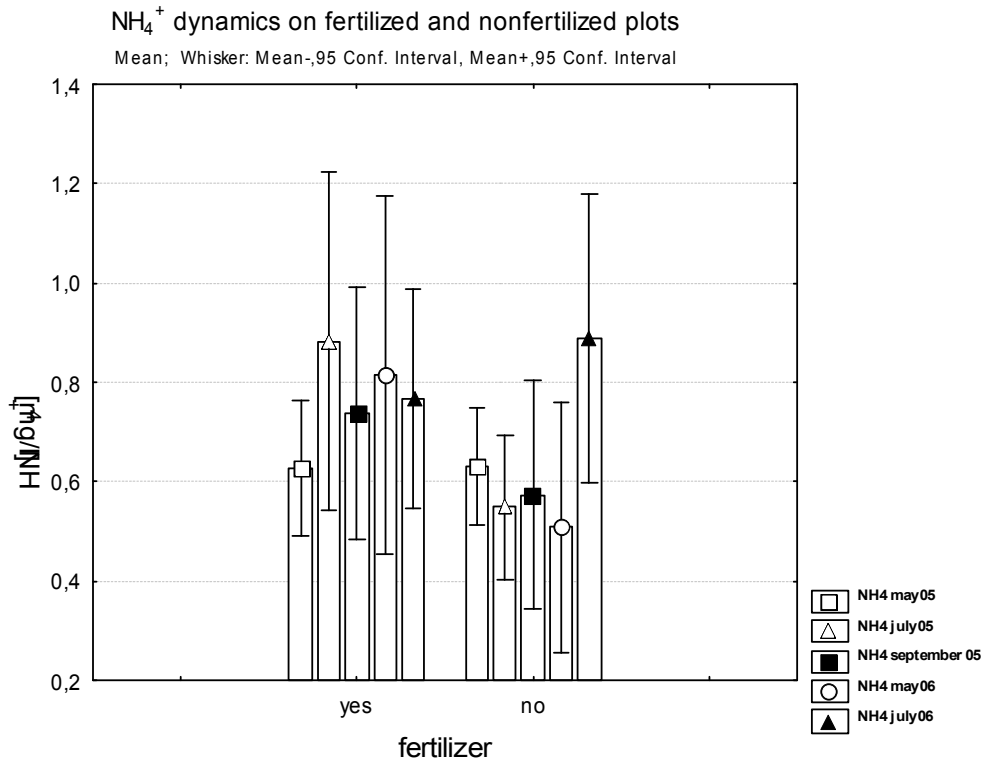


Figure 1. NH<sub>4</sub> dynamics on fertilized and nonfertilized plots

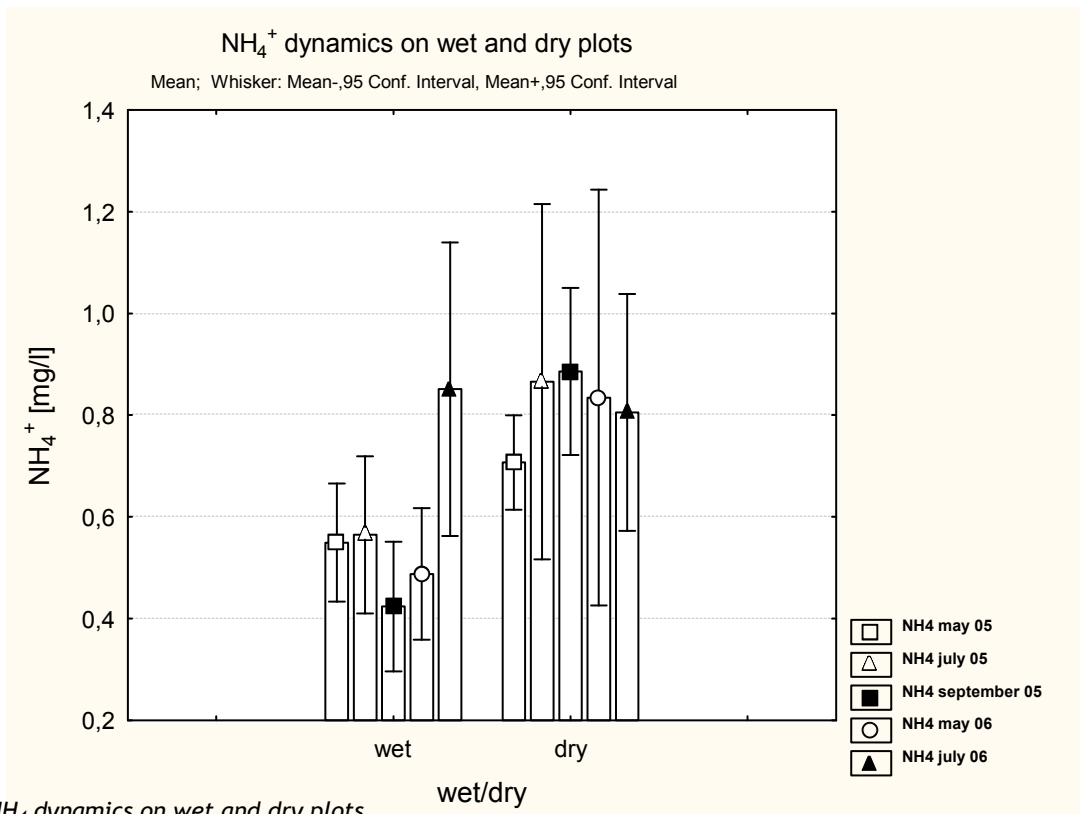


Figure 2. NH<sub>4</sub> dynamics on wet and dry plots

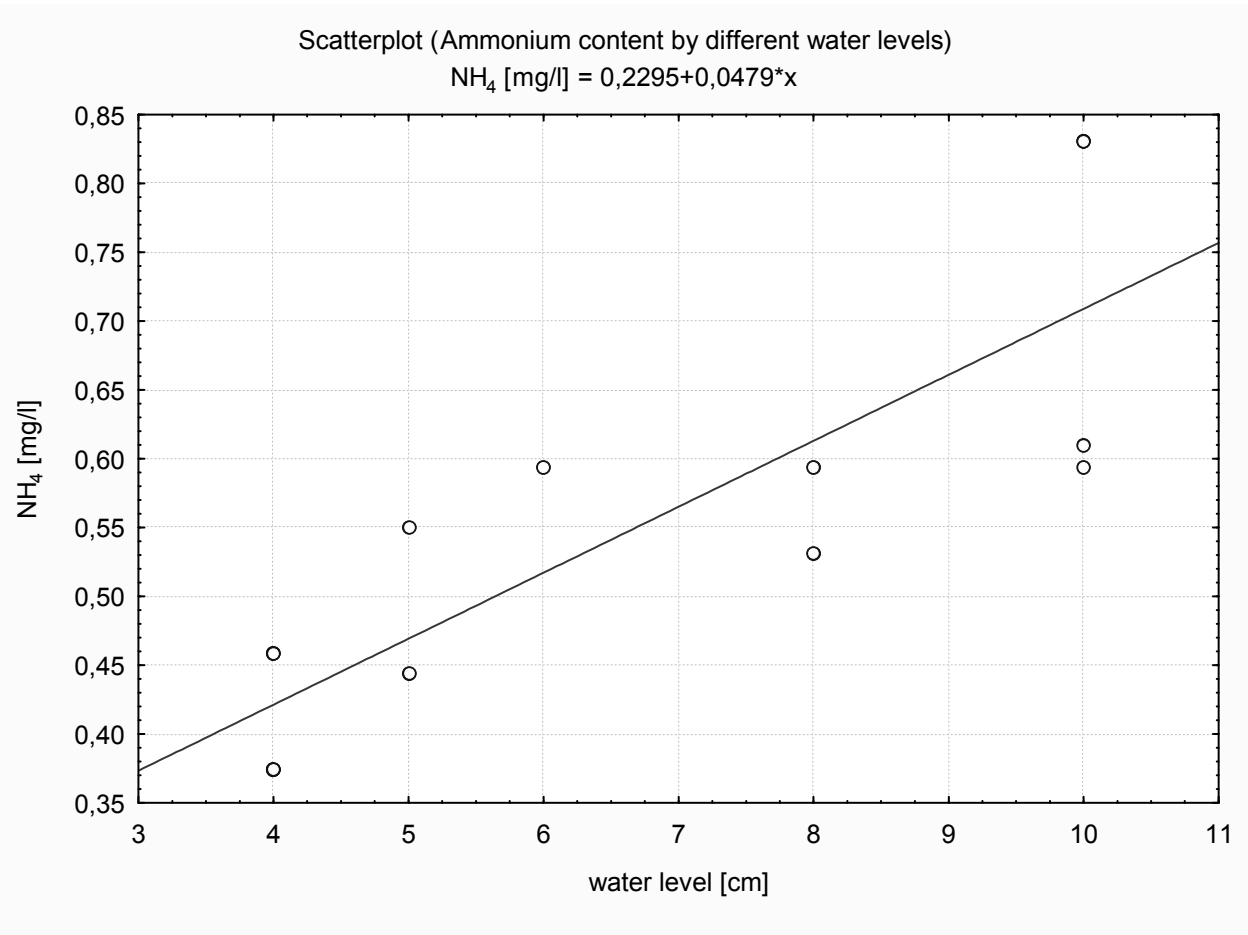


Figure 3. Relationship between water level and NH<sub>4</sub> content

# N-NO<sub>3</sub>

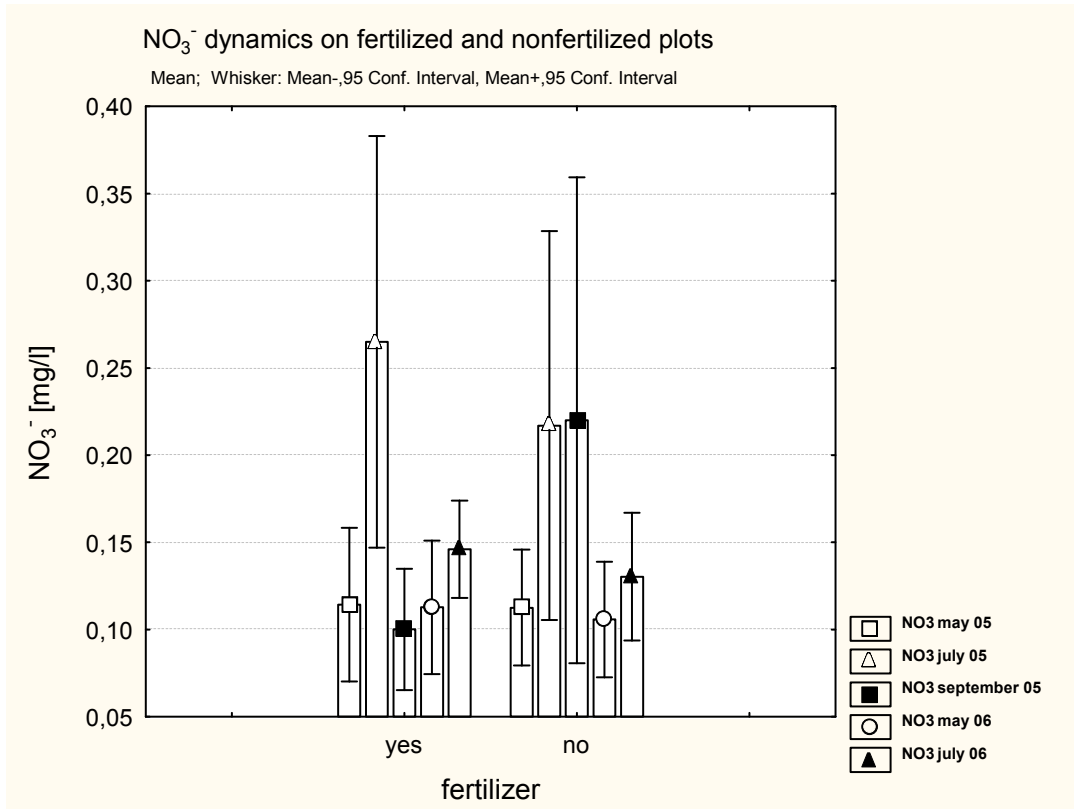


Figure. 5: NO<sub>3</sub> dynamics on fertilized and nonfertilized plots

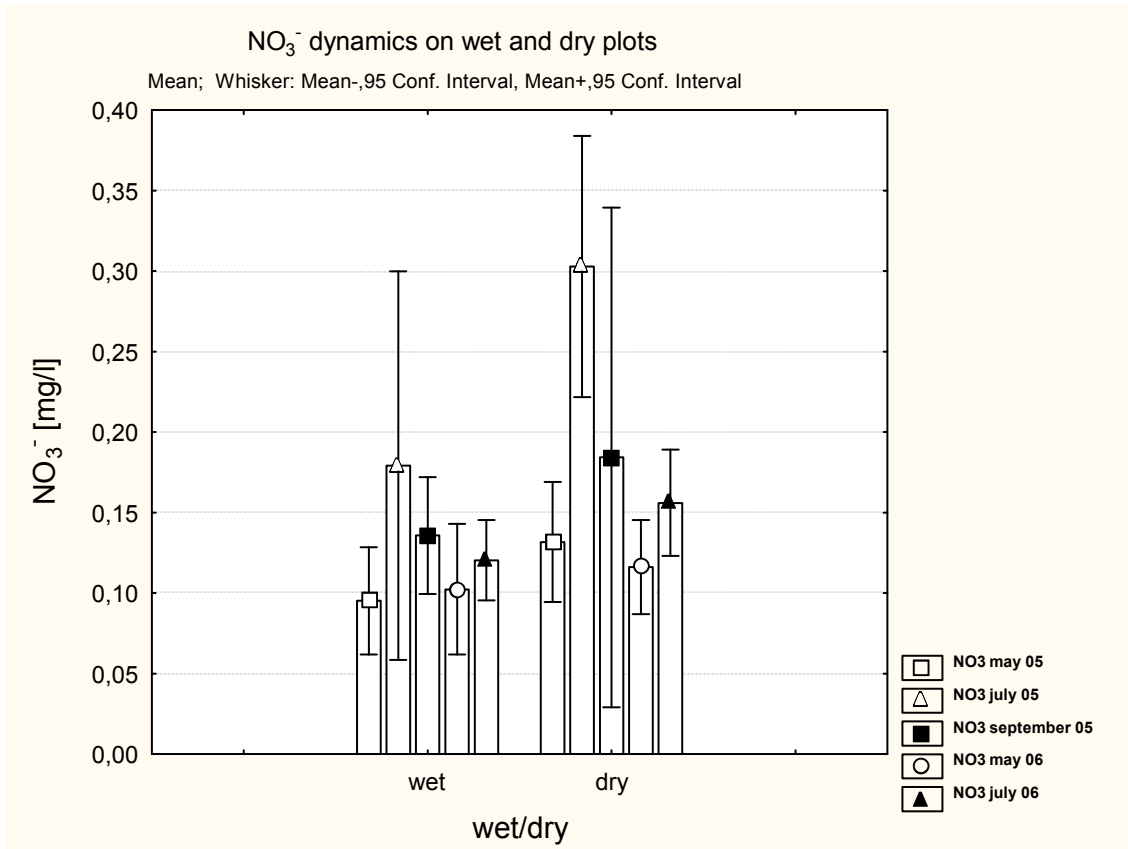


Figure. 6: NO<sub>3</sub> dynamics on wet and dry plots

## Total nitrogen (TN)

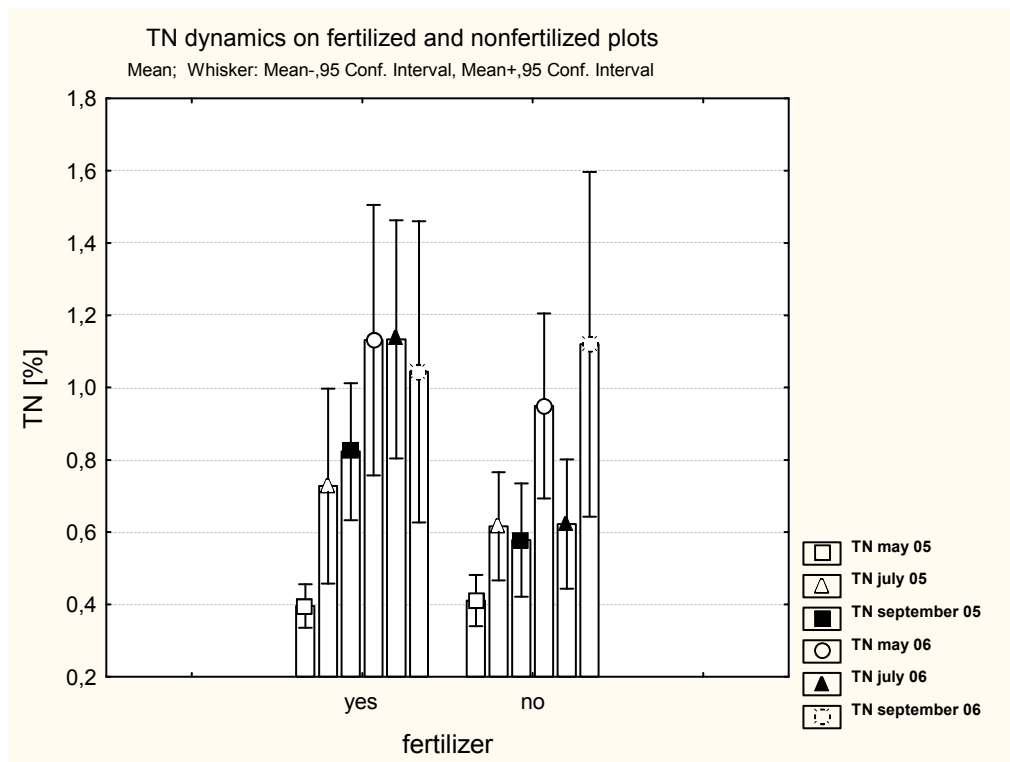


Figure 7: Total nitrogen dynamics on fertilized and nonfertilized plots

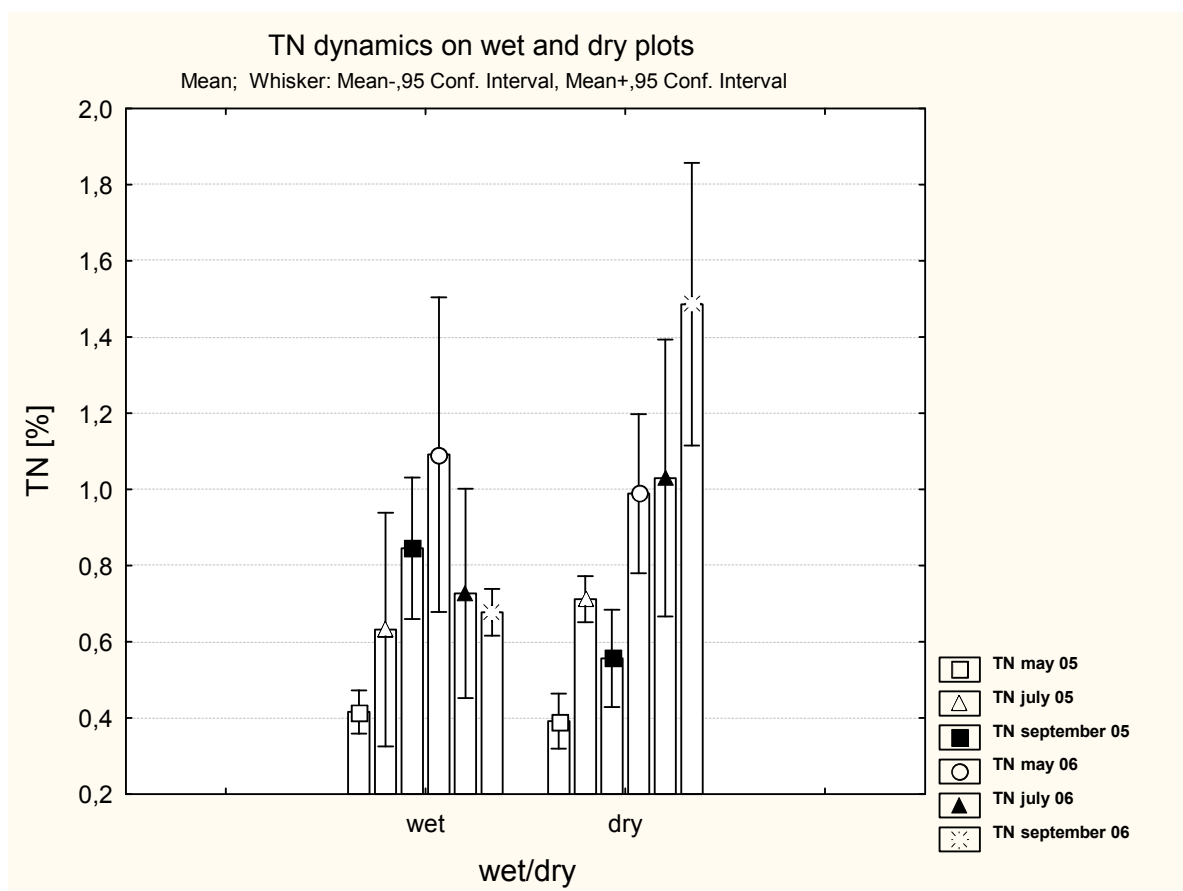


Figure 8: Total nitrogen dynamics on wet and dry plots

## Soluble carbon (C sol)

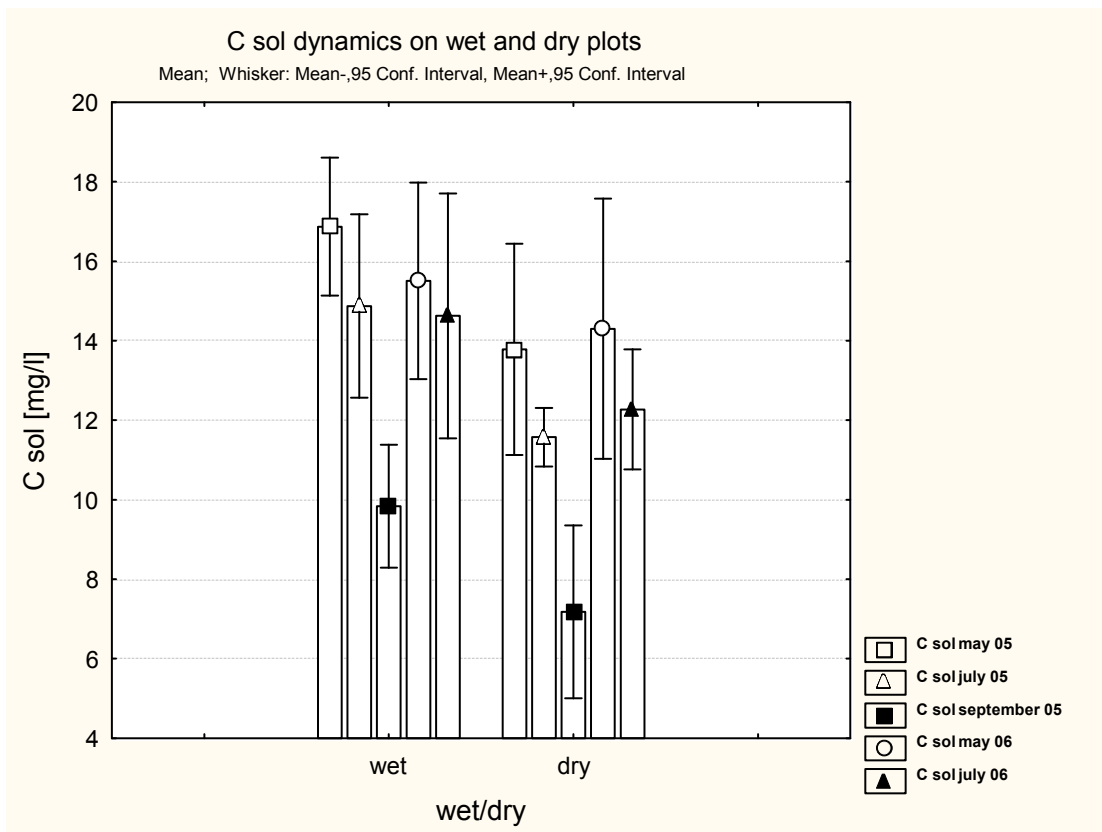


Figure. 9: Soluble carbon dynamics on fertilized and nonfertilized plots

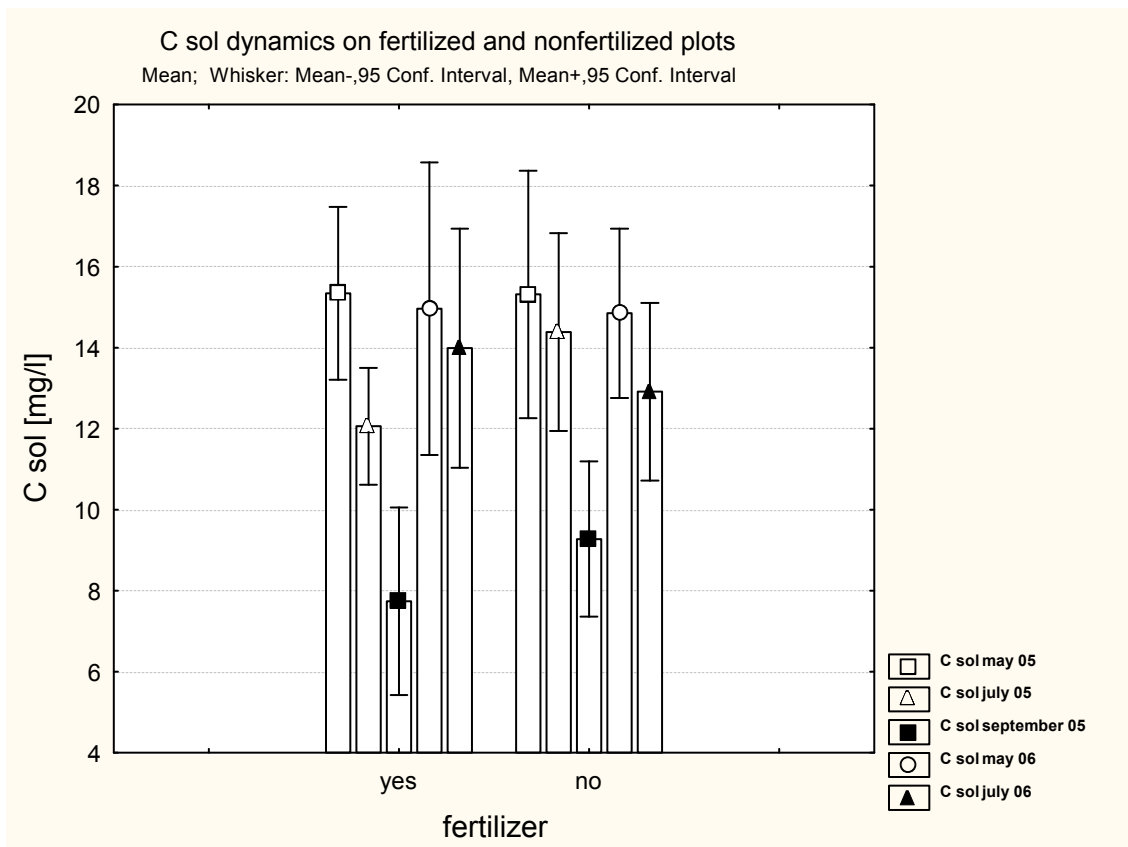


Figure. 10: Soluble carbon dynamics on wet and dry plots



## Total carbon (TC)

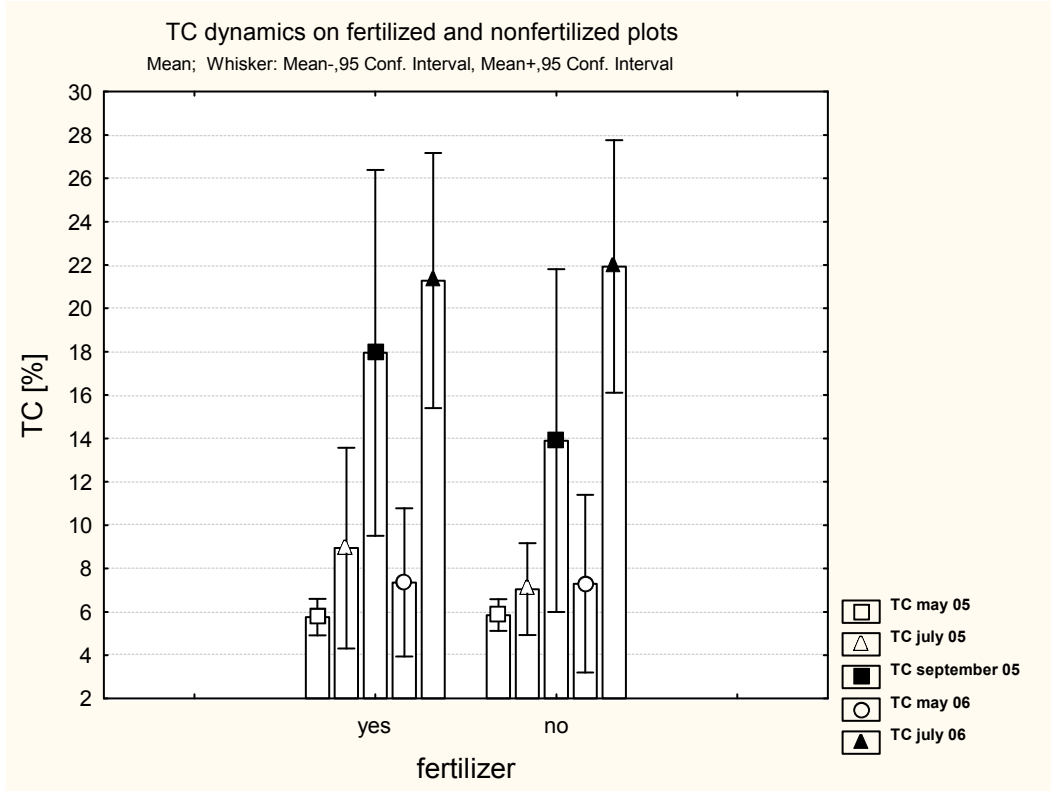


Figure 11: Total carbon dynamics on fertilized and nonfertilized plots

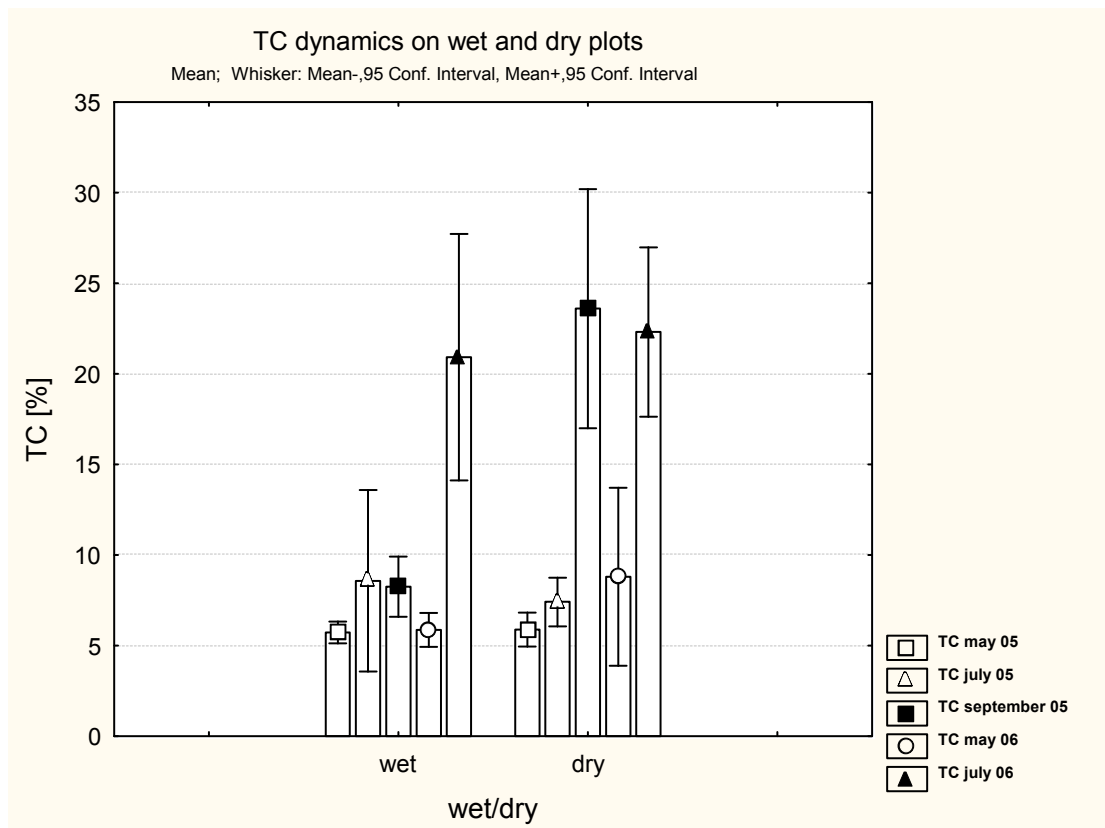


Figure 12: Total carbon dynamics on wet and dry plots

## Total phosphorus (TP) in plant biomass

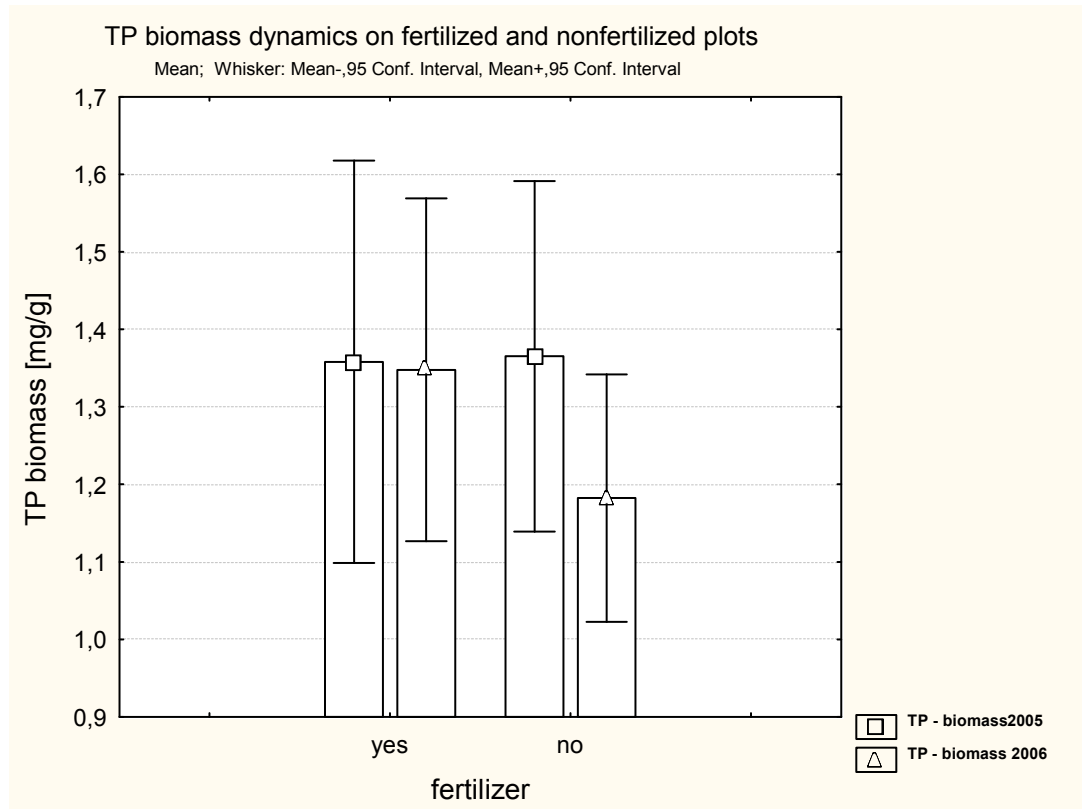


Figure. 13: Total phosphorus dynamics in biomass on fertilizes and nonfertilized plots

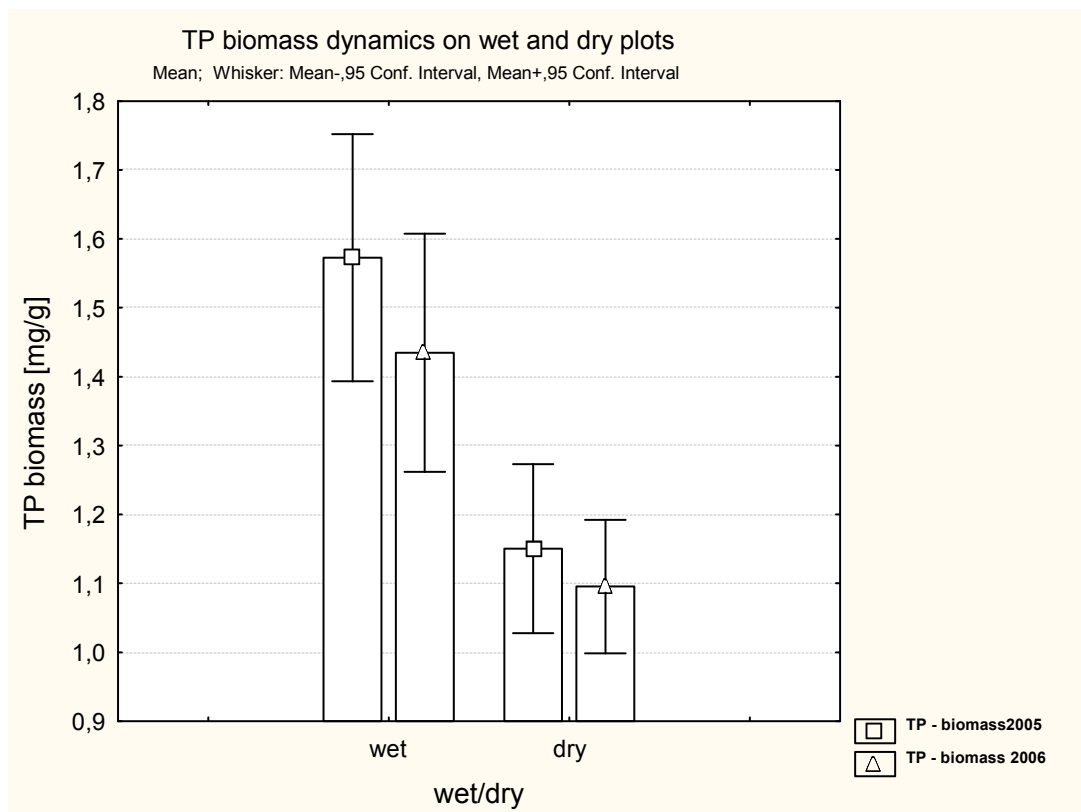


Figure. 14: Total phosphorus dynamics on wet and dry plots -biomass

## Total nitrogen (TN) in plant biomass

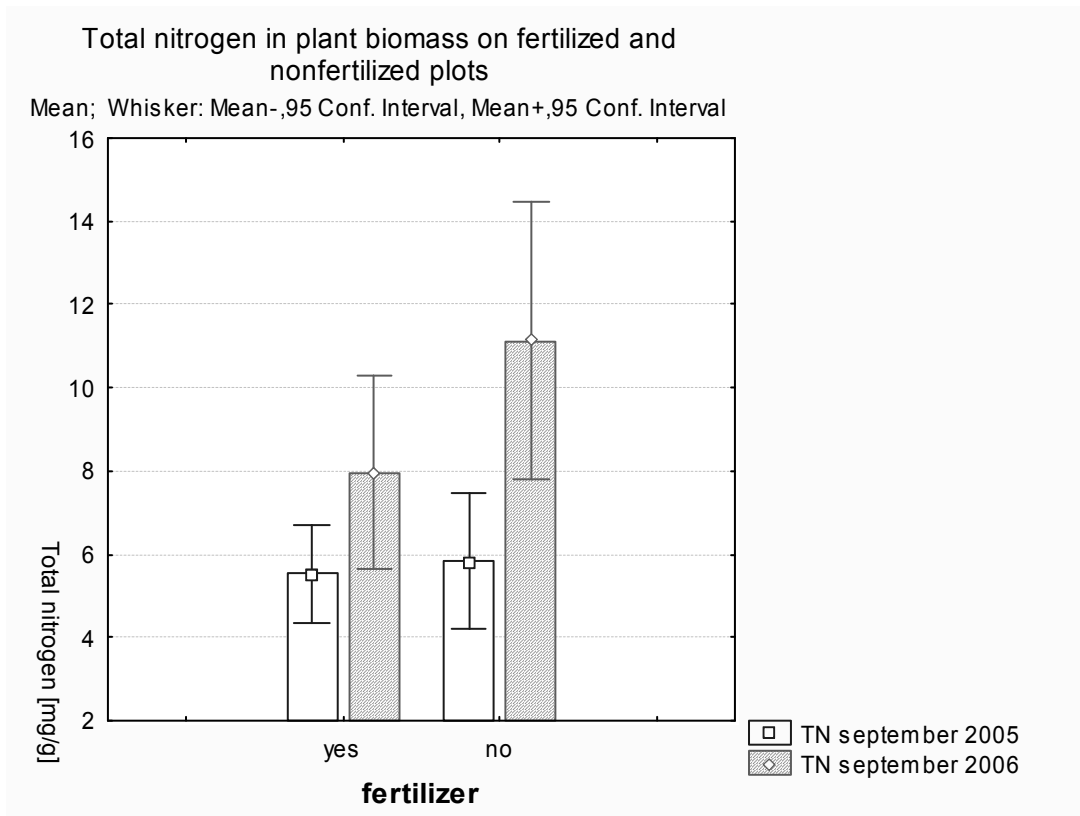


Figure. 15: Total nitrogen in biomass- dynamics on fertilized and nonfertilized plots

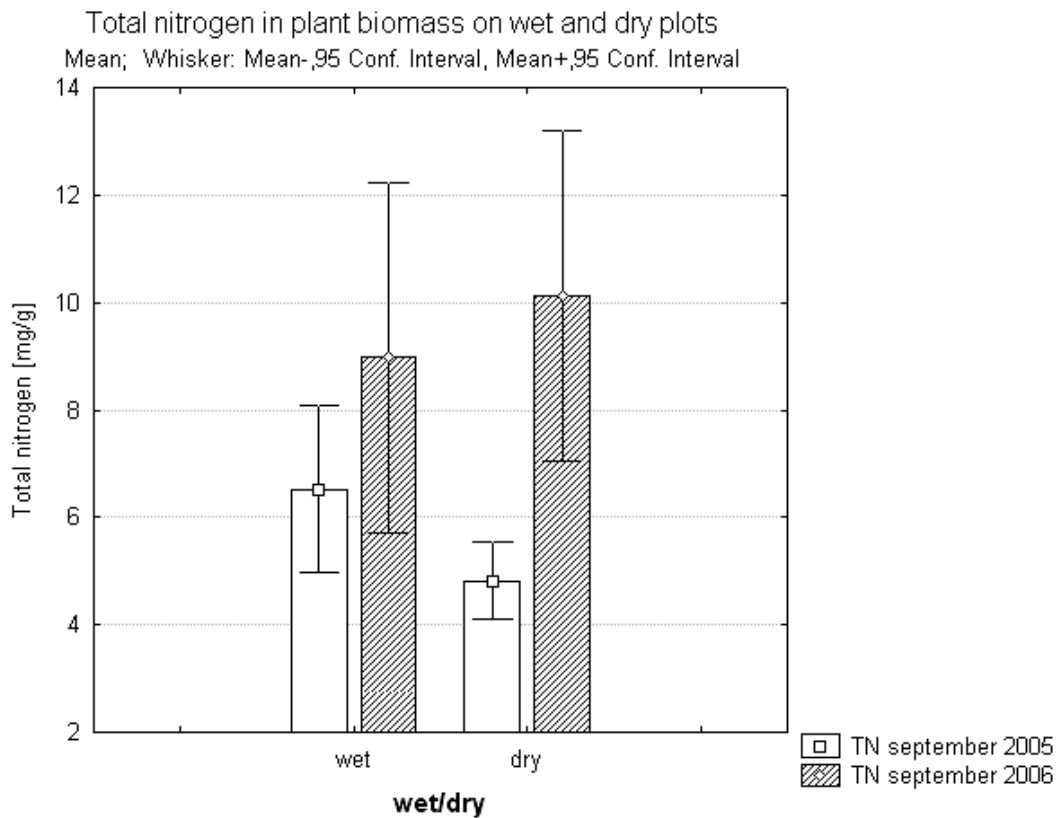


Figure. 16: Total phosphorus in biomass - dynamics on wet and dry plots

## Net aboveground biomass production

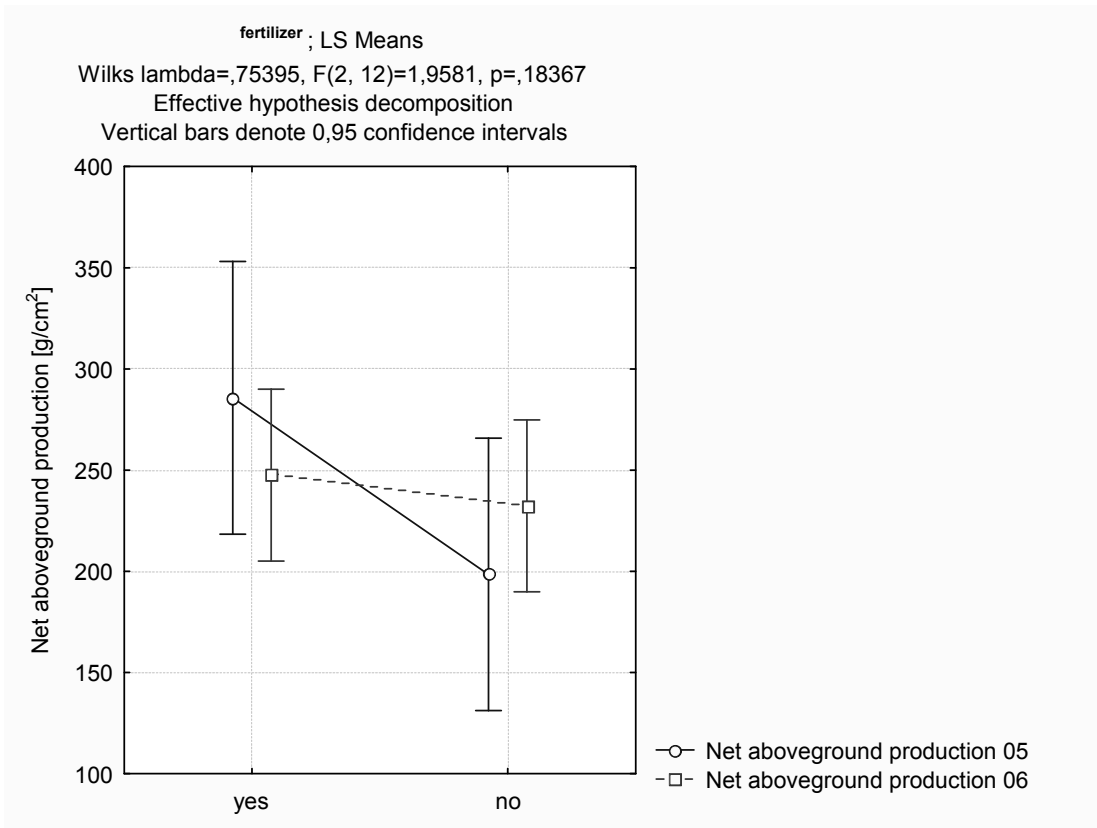


Figure. 17: Net aboveground biomass production on fertilized and nonfertilized plots

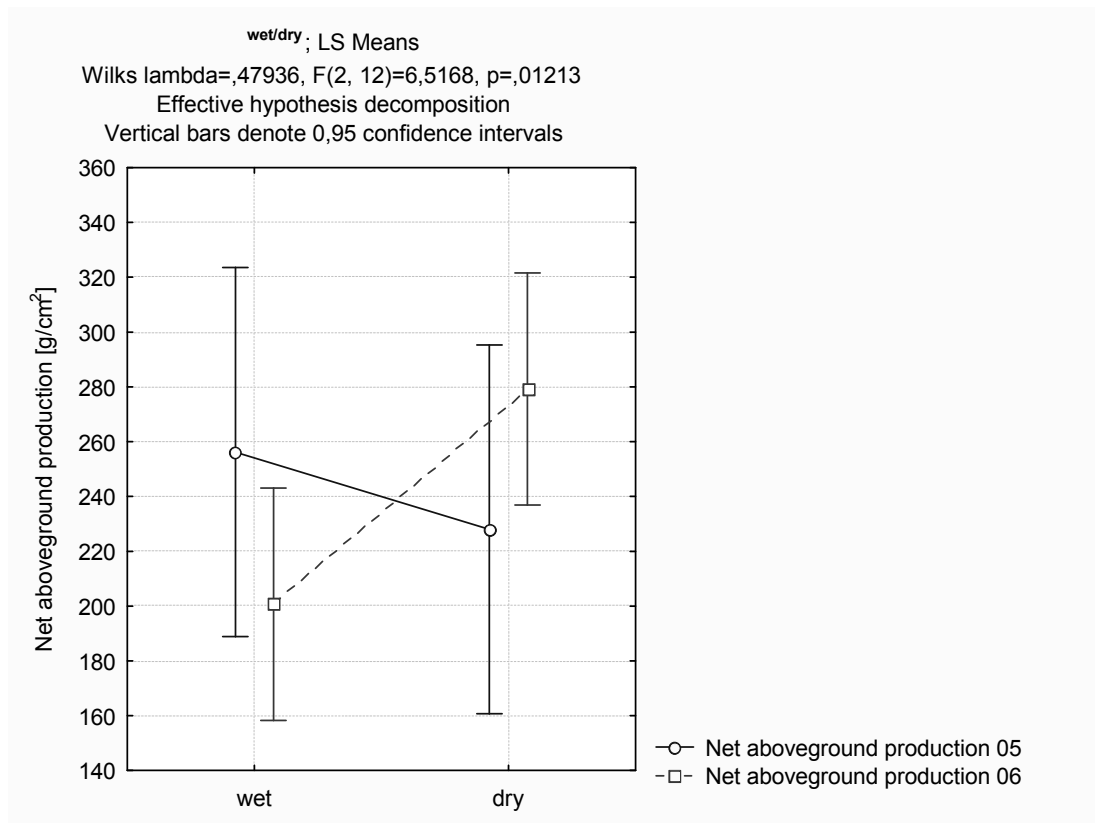


Figure. 18: Net aboveground biomass production on wet and dry plots

## Nitrogen use efficiency

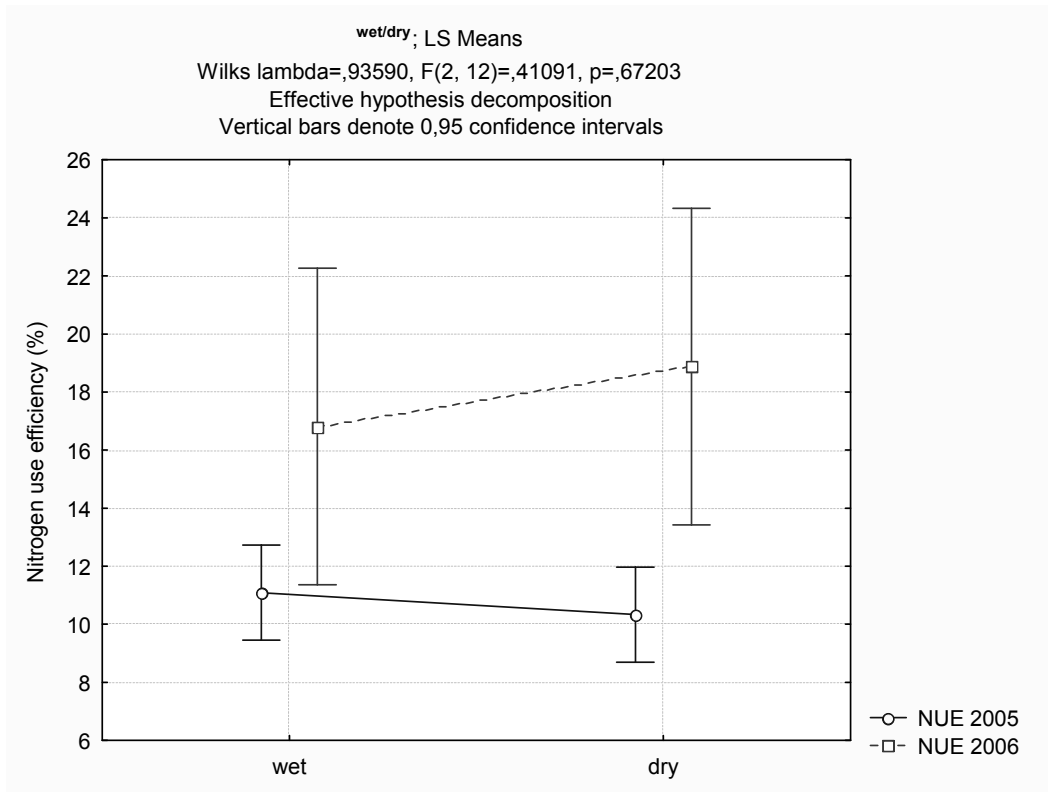


Figure. 19: Nitrogen uptake efficiency on fertilized and nonfertilized plots

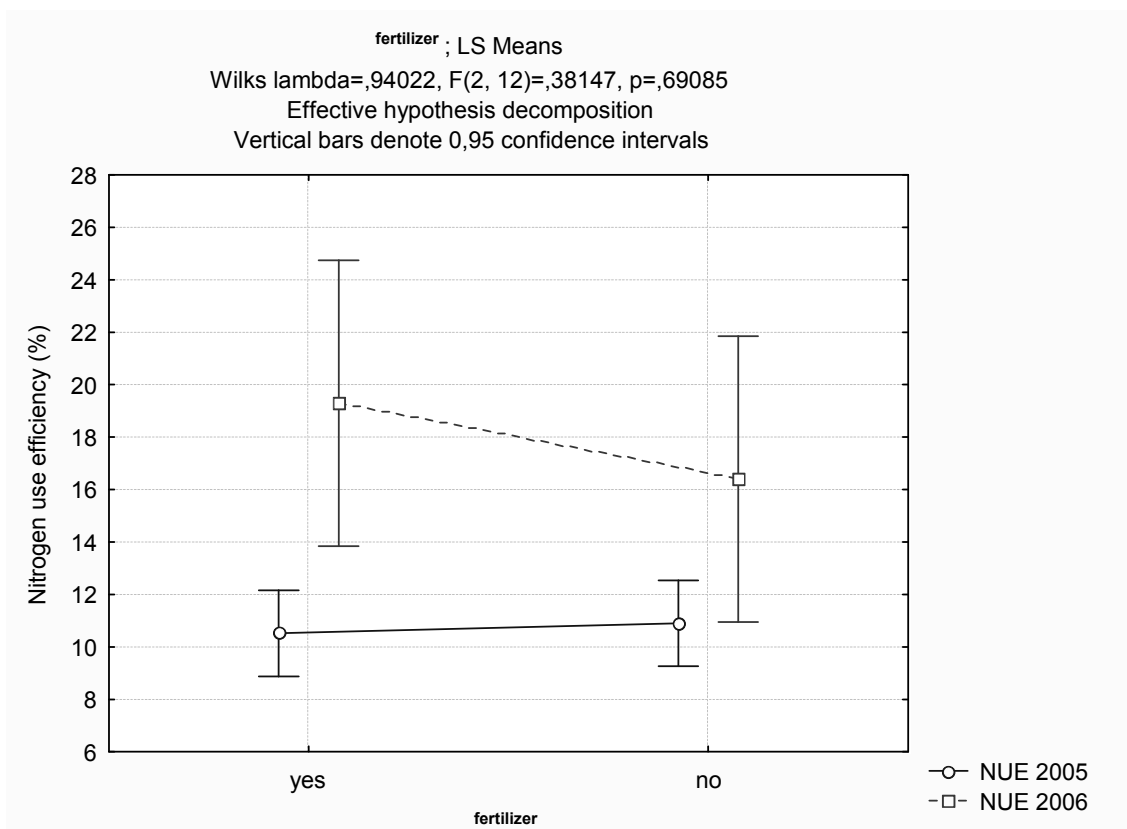


Figure. 20: Nitrogen uptake efficiency on wet and dry plots

## Dry biomass weight

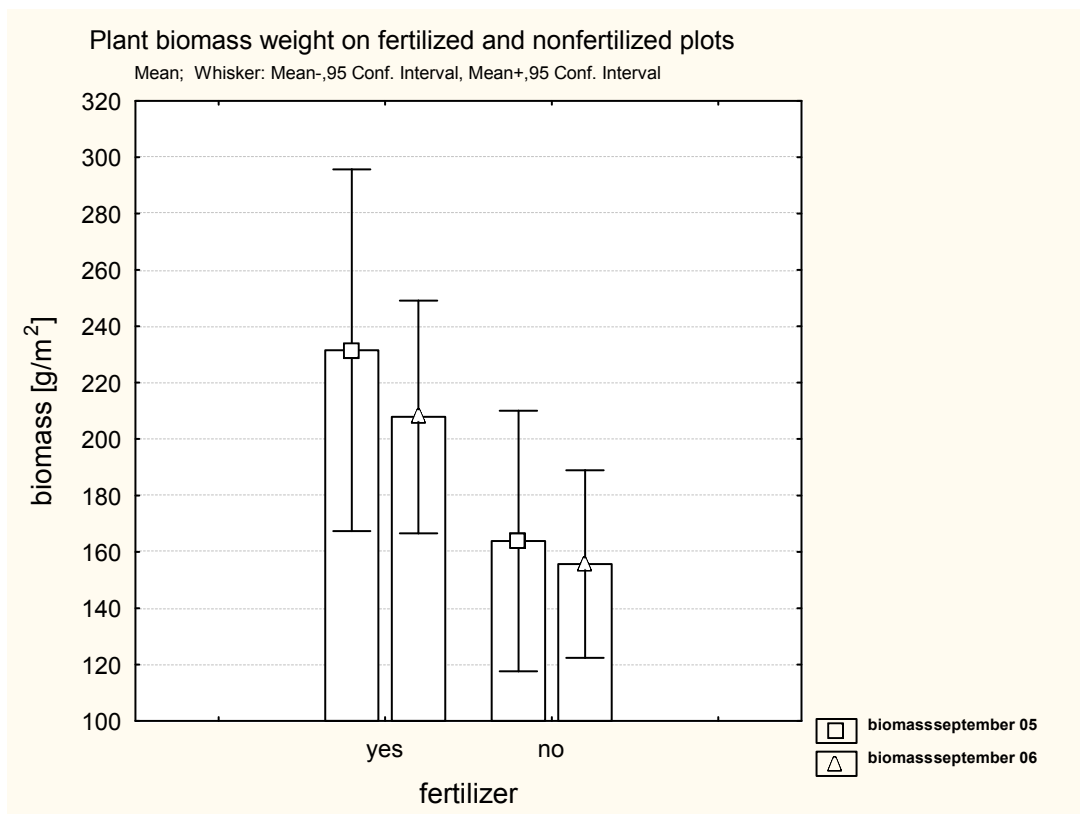


Figure. 21: Biomass weight on fertilized and nonfertilized plots

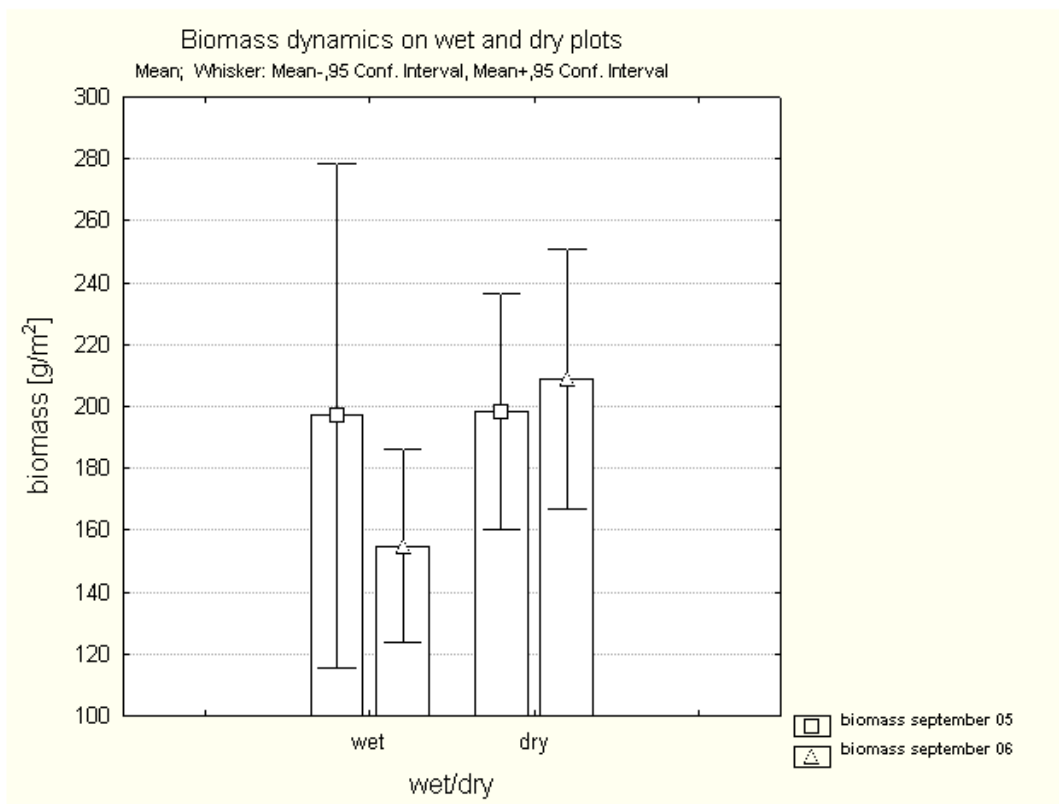


Figure. 22: Biomass weight on wet and dry plots

## Organic matter content

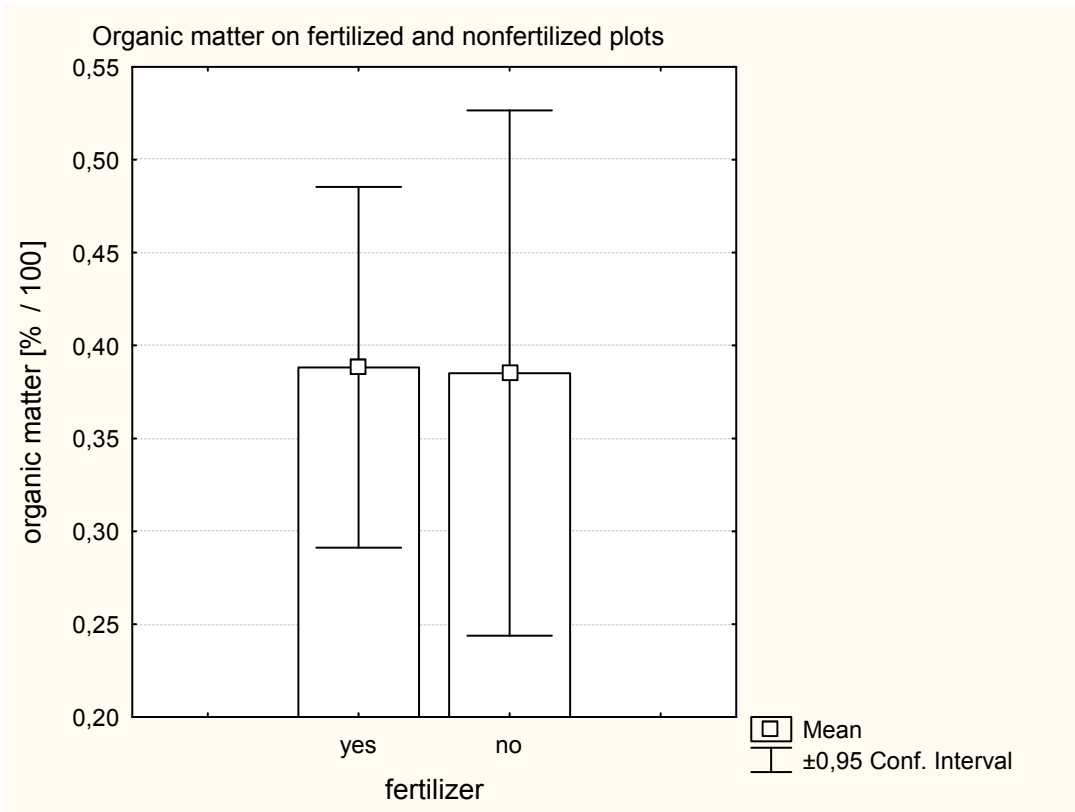


Figure. 23: Organic matter content on fertilized and non fertilized plots

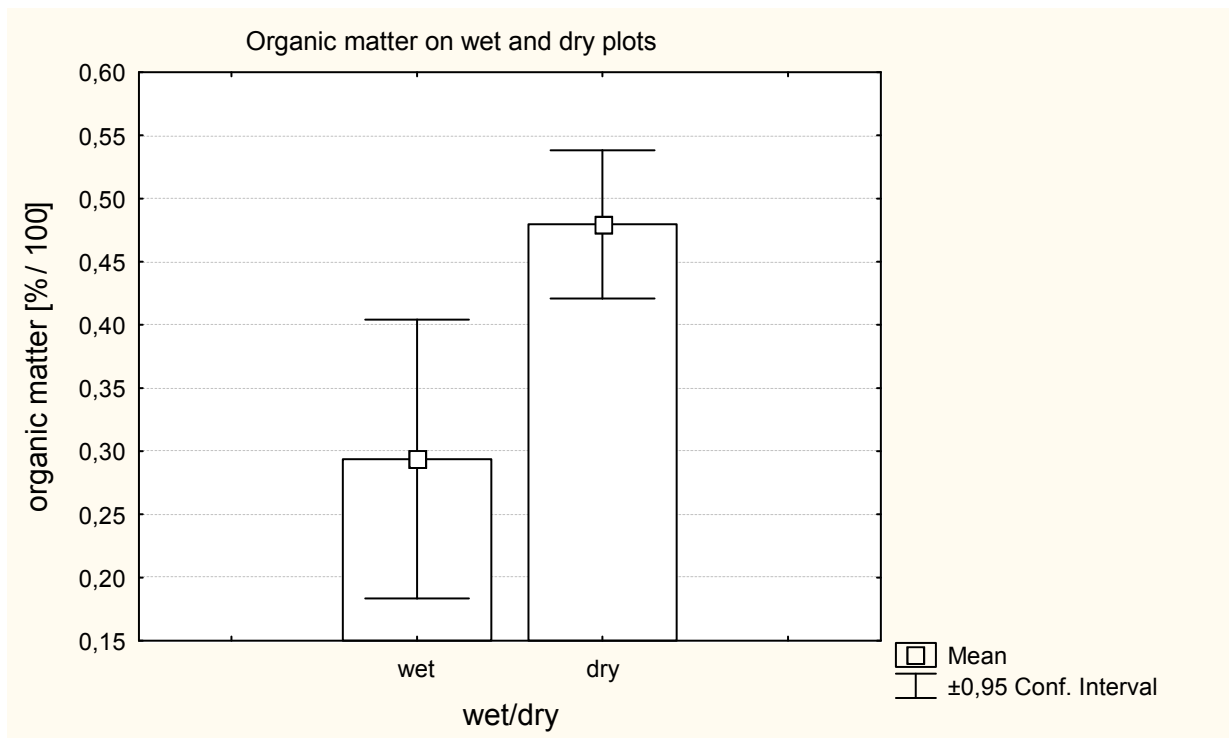


Figure. 24: Organic matter content on wet and dry plots

Záblatské louky and Hamr (Edwards, not published)

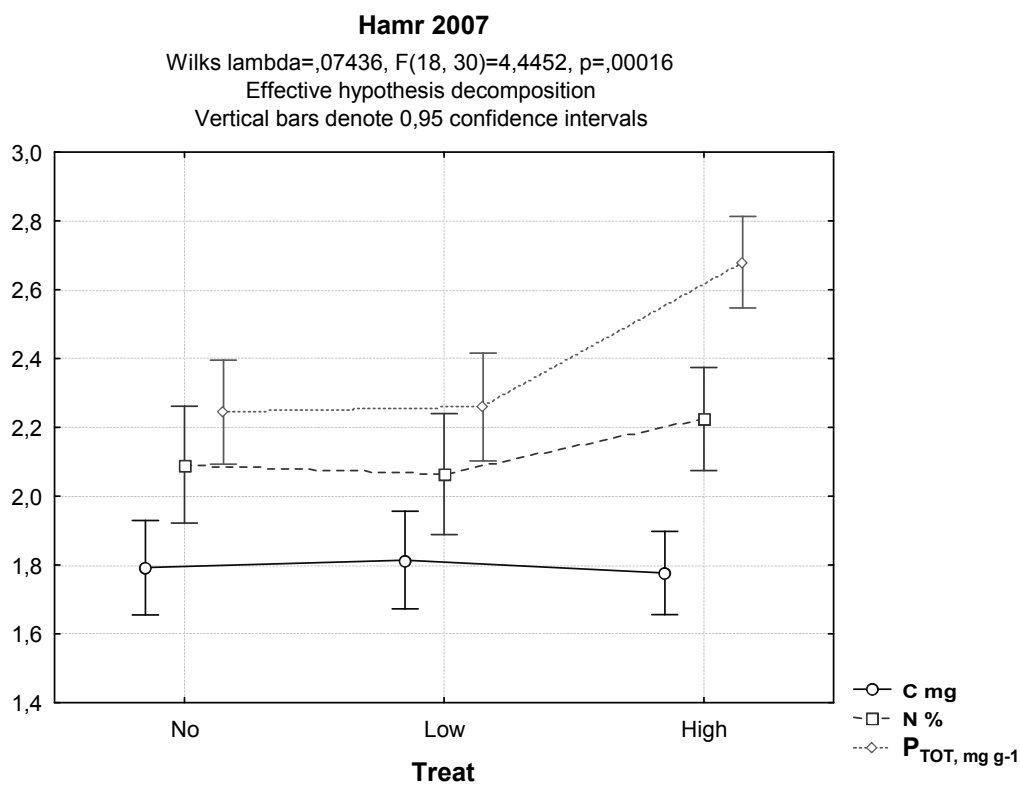


Figure. 25: Nutrient content on fertilized and non fertilized plots on Hamr in 2007

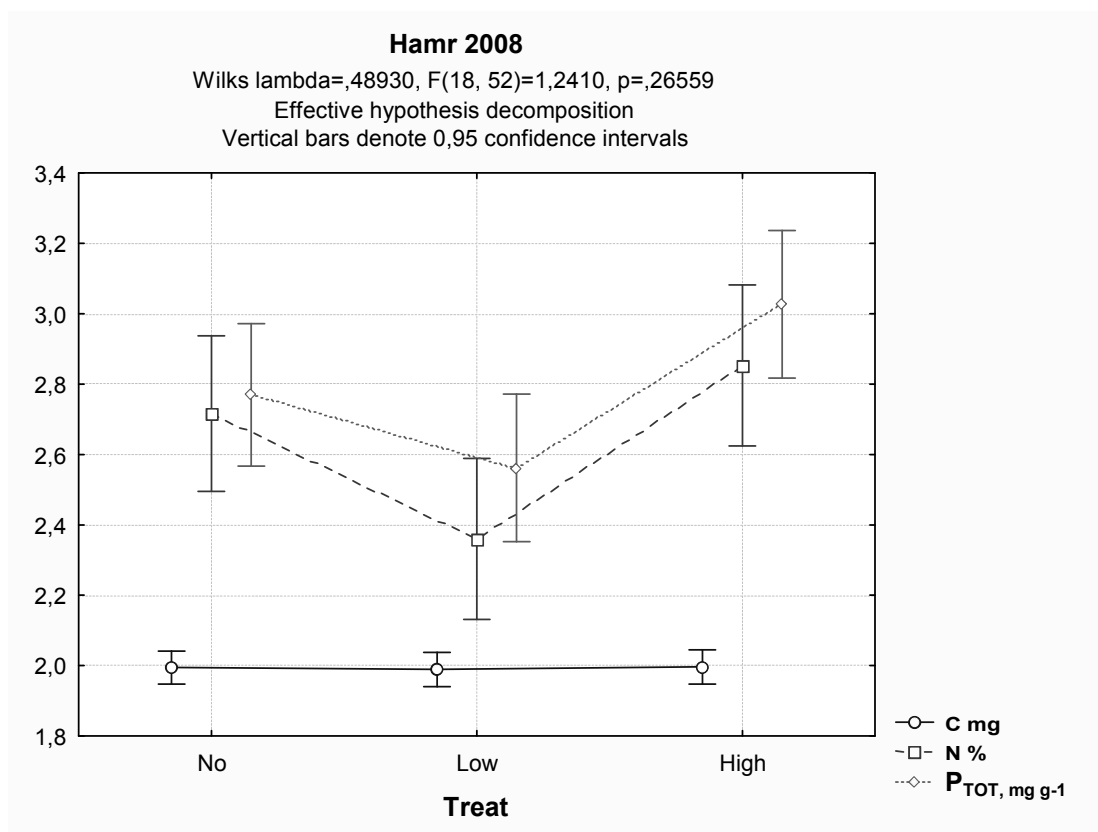


Figure. 26: Nutrient content on fertilized and non fertilized plots on Hamr in 2008



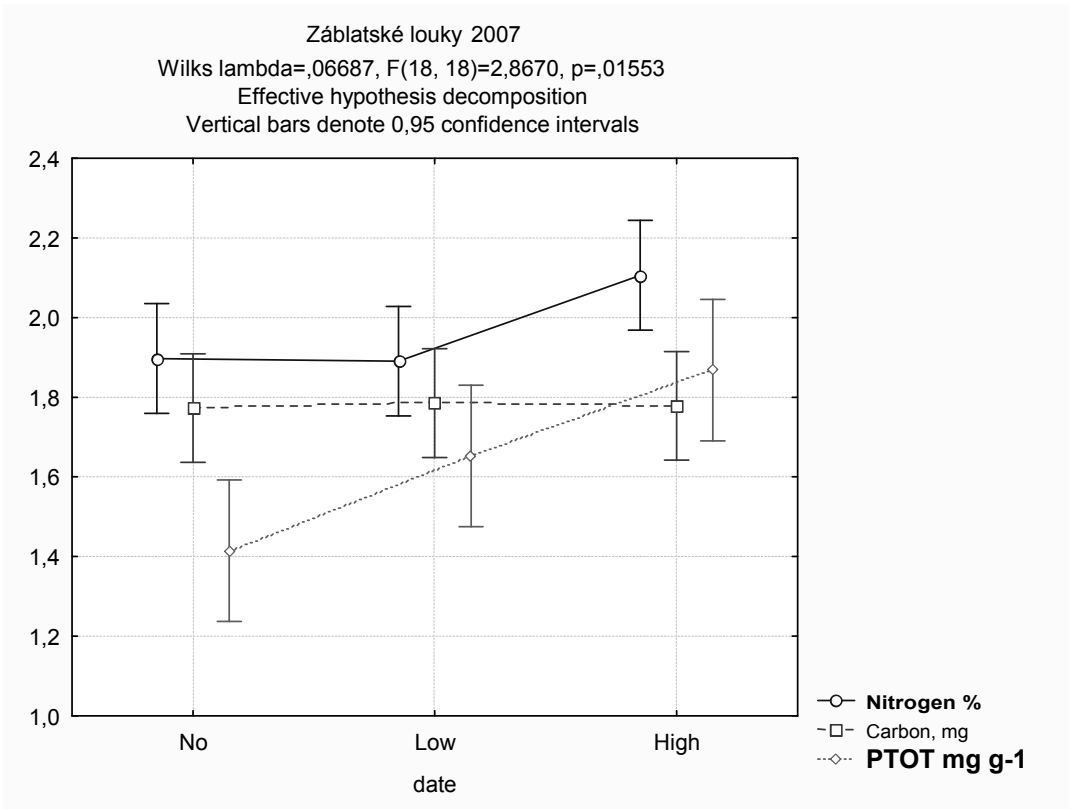


Figure. 27: Nutrient content on fertilized and non fertilized plots on Záblatské louky in 2007

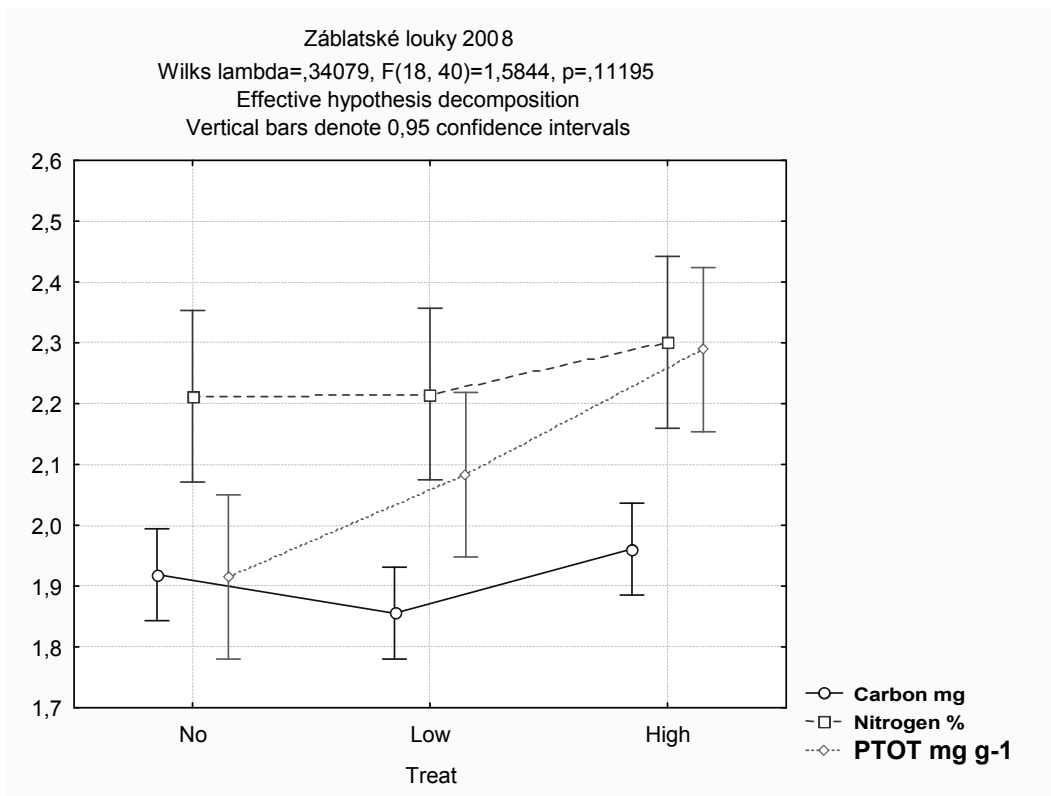


Figure. 28: Nutrient content on fertilized and non fertilized plots on Záblatské louky in 2008

## Sampling design

Black squares represent fertilized plots, white squares represent control plots

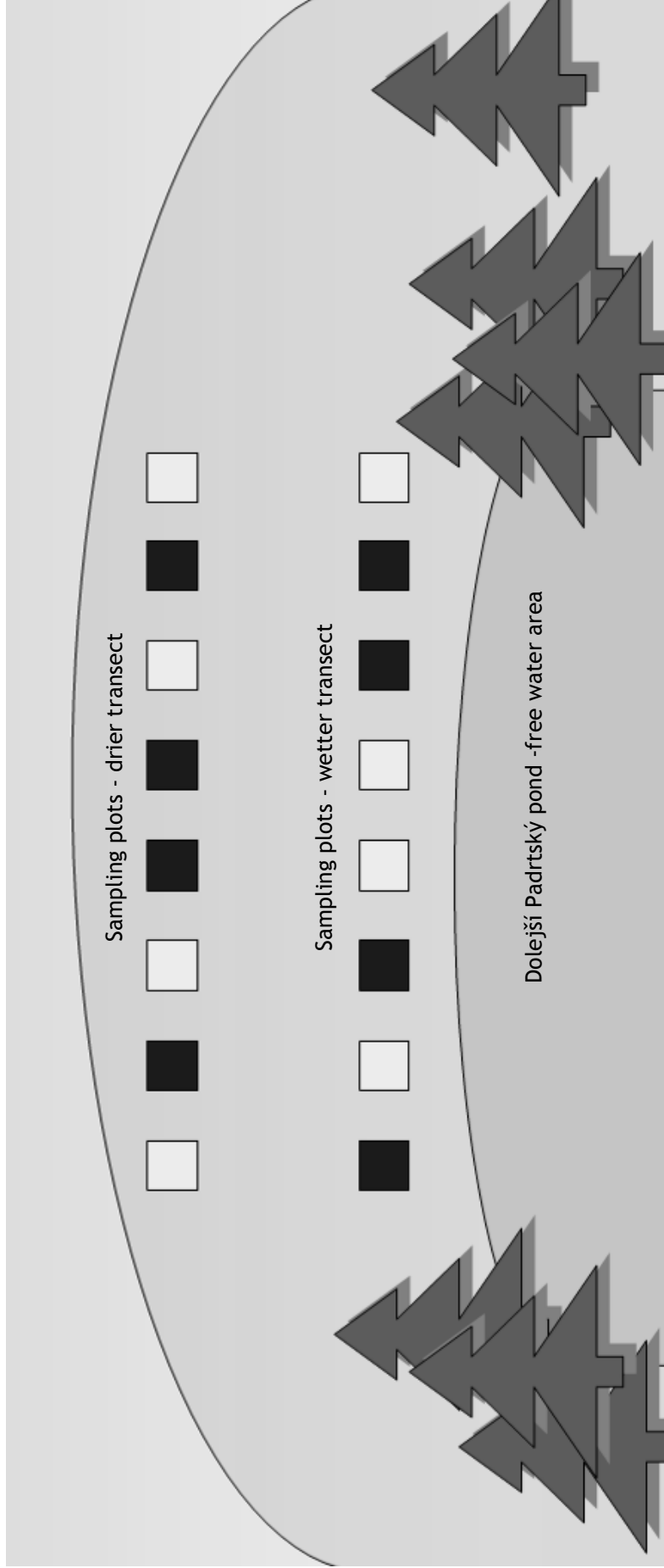


Figure 29: Sampling design

**Study site - a map**



Figure. 30: White circle shows the study site